

FORM PTO-1390 (Modified)
(REV 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER

99,423-S

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

10/018446

INTERNATIONAL APPLICATION NO.
PCT/US00/40281

INTERNATIONAL FILING DATE
21 June 2000

PRIORITY DATE CLAIMED
22 June 1999

TITLE OF INVENTION

N-Pyrazole A2A Receptor Agonists

APPLICANT(S) FOR DO/EO/US

Jeff Zablocki et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ has been communicated by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☐ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☒ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Patent Data Sheet

U.S. APPLICATION NO. (UNKNOWN) 37 CFR 107/018446

INTERNATIONAL APPLICATION NO.

PCT/US00/40281

ATTORNEY'S DOCKET NUMBER

99,423-S

24. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

- ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1040.00
- ☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00
- ☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00
- ☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00
- ☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

\$890.00

Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)) ☐ 20 ☒ 30

\$130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total claims	24 - 20 =	4	x \$18.00
Independent claims	1 - 3 =	0	x \$80.00
Multiple Dependent Claims (check if applicable).			<input type="checkbox"/>

\$72.00

\$0.00

\$0.00

TOTAL OF ABOVE CALCULATIONS =

\$1,092.00

☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.

\$0.00

SUBTOTAL =

\$1,092.00

Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)). ☐ 20 ☐ 30 +

\$0.00

TOTAL NATIONAL FEE =

\$1,092.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). ☐

\$0.00

TOTAL FEES ENCLOSED =

\$1,092.00

Amount to be:
refunded

\$

charged

\$

- a. ☒ A check in the amount of \$1,092.00 to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-2490. A duplicate copy of this sheet is enclosed.
- d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

A. Blair Hughes

NAME

32,901

REGISTRATION NUMBER

December 13, 2001

DATE

100013446 107018446

531 Rec'd PCT/PT 13 DEC 2001

CERTIFICATE OF MAILING BY "EXPRESS MAIL"
(NEW PATENT APPLICATION)

Express Mail No. EL604655725US

Deposited December 13, 2001

I hereby certify that the attached correspondence, identified below, is being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" under 37 CFR § 1.10 on the date indicated above and is addressed to the Box PCT, Commissioner for Patents, Washington, DC 20231.

By: James Walker
(person actually depositing)

Patent Application of: Jeff Zablocki, et al.

Title: N-Pyrazole A2A Receptor Agonists

X Transmittal Letter to the United States Designated/Elected Office (DO/EO/US)

X Postcard

X Copy of International Search Report

X Check

X Copy of International Preliminary Examination Report

X Patent Data Sheet

Attorney Docket No.: 99,423-S

TITLE: N-Pyrazole A_{2A} Receptor Agonists

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Background Of The Invention

Field of Invention

This invention includes N-pyrazole substituted 2-adenosine compounds that are useful as A_{2A} receptor agonists. The compounds of this invention are vasodilating agents that are useful as heart imaging aids that aid in the identification of mammals, and especially humans who are suffering from coronary disorders such poor coronary perfusion which is indicative of coronary artery disease (CAD). The compounds of this invention can also be used as therapeutics for coronary artery disease as well as any other disorders mediated by the A_{2A} receptor.

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Description of the Art

Pharmacological stress is frequently induced with adenosine or dipyridamole in patients with suspected CAD before imaging with Tl scintigraphy or echocardiography. Both drugs effect dilation of the coronary resistance vessels by activation of cell surface A₂ receptors. Although pharmacological stress was originally introduced as a mean of provoking coronary dilation in patients unable to exercise, several studies have shown that the prognostic value of ²⁰¹Tl or echocardiographic imaging in patients subjected to pharmacological stress with adenosine or dipyridamole was equivalent to patients subjected to traditional exercise stress tests. However, there is a high incidence of drug-related adverse side effects during pharmacological stress imaging with these drugs such as headache and nausea, that could be improved with new therapeutic agents.

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Adenosine A_{2B} and A₃ receptors are involved in a mast cell degranulation and, therefore, asthmatics are not give the non-specific adenosine agonists to induce a pharmacological stress test. Additionally, adenosine stimulation of the A₁ receptor in the atrium and A-V node will diminish the S-H interval which can induce AV block (N.C. Gupta et al.; *J. Am Coll. Cardiol*; (1992) 19: 248-257). Also, stimulation of the adenosine A₁ receptor by adenosine may be responsible for the nausea since the A₁ receptor is found in the intestinal tract (J. Nicholls et al.; *Eur. J. Pharm.*(1997) 338(2) 143-150).

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Animal data suggests that specific adenosine A_{2A} subtype receptors on coronary resistance vessels mediate the coronary dilatory responses to adenosine, whereas subtype A_{2B} receptor stimulation relaxes peripheral vessels (note: the latter lowers systemic blood

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pressure). As a result there is a need for pharmaceutical compositions that are A_{2A} receptor agonists that have no pharmacological effect as a result of stimulating the A_1 receptor *in vivo*. Furthermore, there is a need for A_{2A} receptor agonists that have a short half-life, and that are well tolerated by patients undergoing pharmacological coronary stress evaluations.

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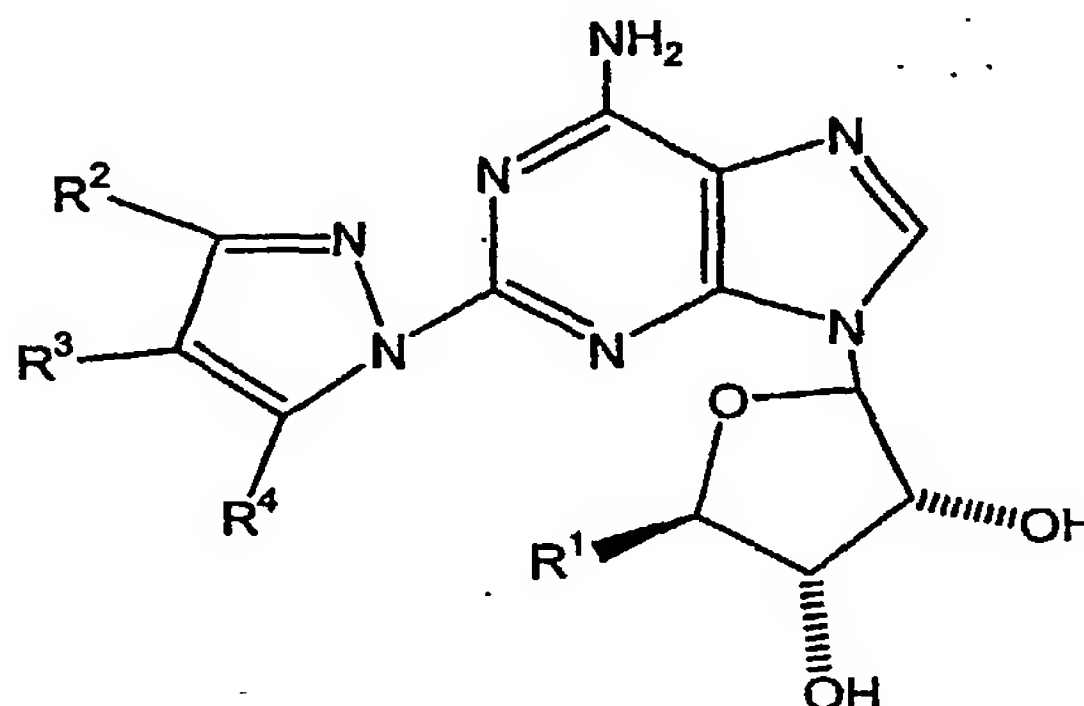
SUMMARY OF THE INVENTION

In one aspect, this invention includes 2-adenosine N-pyrazole compounds that are useful A_{2A} receptor agonists.

10 In another aspect, this invention includes pharmaceutical compounds including 2-adenosine N-pyrazole that are well tolerated with few side effects.

Still another aspect of this invention are N-pyrazole compounds that can be easily used in conjunction with radioactive imaging agents to facilitate coronary imaging.

In one embodiment, this invention includes 2-adenosine N-pyrazole compounds having the following formula:



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In another embodiment, this invention includes methods for using compounds of this invention to stimulate coronary vasodilatation in mammals, and especially in humans, for stressing the heart induced steal situation for purposes of imaging the heart.

20 In still another embodiment, this invention is a pharmaceutical composition comprising one or more compounds of this invention and one or more pharmaceutical excipients.

DESCRIPTION OF THE FIGURES

Figure 1A is a analog record of the increase in coronary conductance caused by Compound 16 of this invention before and after infusions of CPX and ZM241385;

5 Figure 1B is a summary of the data shown in Figure 1A showing that CPX did not but that ZM241385 did attenuate the increase in coronary conductance caused by Compound 16 of this invention. In Figure 1B, the bars represent mean \pm SEM of single measurement from 6 rat isolated perfused hearts;

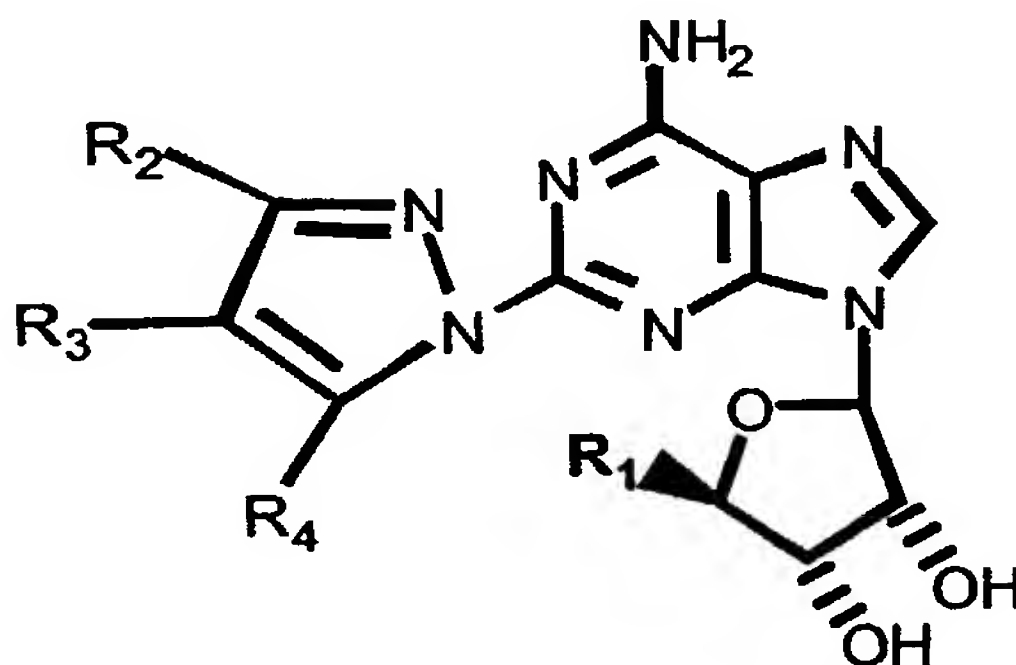
10 Figure 2 is a concentration response curve for the A_1 adenosine receptor (AdoR)-mediated negative dromotropic (AV conduction time) and A_{2A} AdoR-mediated vasodialator (increase coronary conductance) effects of Compound 16 in rat isolated perfused hearts. Symbols and error bars indicate means \pm SEM of single determination from each of four hearts. EC_{50} value (potency) is the concentration of Compound 16 that causes 50% of maximal response;

15 Figure 3 is a concentration response curve for the A_1 adenosine receptor (AdoR)-mediated negative dromotropic (AV conduction time) and A_{2A} AdoR-mediated vasodialator (increase coronary conductance) effects of Compound 16 in guinea pig isolated perfused hearts. Symbols and error bars indicate means \pm SEM of single determination from each of four hearts. EC_{50} value (potency) is the concentration of Compound 16 that causes 50% of
20 maximal response; and

Figure 4 is a plot of the effect of CVT510, an A_1 adenosine receptor agonist and Compound 16 of this invention, an A_{2A} adenosine receptor agonist on atrioventricular (AV) conduction time in rat isolated perfused hearts.

DESCRIPTION OF THE CURRENT EMBODIMENT

This invention includes a class of 2-adenosine N-pyrazole having the formula:



wherein $R^1 = \text{CH}_2\text{OH}$, $-\text{CONR}_5\text{R}_6$;

R^3 is independently selected from the group consisting of C_{1-15} alkyl, halo, NO_2 , CF_3 , CN , OR^{20} , SR^{20} , $\text{N}(\text{R}^{20})_2$, $\text{S}(\text{O})\text{R}^{22}$, SO_2R^{22} , $\text{SO}_2\text{N}(\text{R}^{20})_2$, $\text{SO}_2\text{NR}^{20}\text{COR}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CON}(\text{R}^{20})_2$, $\text{N}(\text{R}^{20})_2\text{NR}^{20}\text{COR}^{22}$, $\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{NR}^{20}\text{CON}(\text{R}^{20})_2$, $\text{NR}^{20}\text{C}(\text{NR}^{20})\text{NHR}^{23}$, COR^{20} , CO_2R^{20} , $\text{CON}(\text{R}^{20})_2$, $\text{CONR}^{20}\text{SO}_2\text{R}^{22}$, $\text{NR}^{20}\text{SO}_2\text{R}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{OCONR}^{20}\text{SO}_2\text{R}^{22}$, $\text{OC}(\text{O})\text{R}^{20}$, $\text{C}(\text{O})\text{OCH}_2\text{OC}(\text{O})\text{R}^{20}$, and $\text{OCON}(\text{R}^{20})_2$, $-\text{CONR}^7\text{R}^8$, C_{2-15} alkenyl, C_{2-15} alkynyl, heterocyclyl, aryl, and heteroaryl, wherein the alkyl, alkenyl, alkynyl, aryl, heterocyclyl and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, alkyl, NO_2 , heterocyclyl, aryl, heteroaryl, CF_3 , CN , OR^{20} , SR^{20} , $\text{N}(\text{R}^{20})_2$, $\text{S}(\text{O})\text{R}^{22}$, SO_2R^{22} , $\text{SO}_2\text{N}(\text{R}^{20})_2$, $\text{SO}_2\text{NR}^{20}\text{COR}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CON}(\text{R}^{20})_2$, $\text{N}(\text{R}^{20})_2\text{NR}^{20}\text{COR}^{22}$, $\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{NR}^{20}\text{CON}(\text{R}^{20})_2$, $\text{NR}^{20}\text{C}(\text{NR}^{20})\text{NHR}^{23}$, COR^{20} , CO_2R^{20} , $\text{CON}(\text{R}^{20})_2$, $\text{CONR}^{20}\text{SO}_2\text{R}^{22}$, $\text{NR}^{20}\text{SO}_2\text{R}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{OCONR}^{20}\text{SO}_2\text{R}^{22}$, $\text{OC}(\text{O})\text{R}^{20}$, $\text{C}(\text{O})\text{OCH}_2\text{OC}(\text{O})\text{R}^{20}$, and $\text{OCON}(\text{R}^{20})_2$ and wherein the optional substituted heteroaryl, aryl, and heterocyclyl substituents are optionally substituted with halo, NO_2 , alkyl, CF_3 , amino, mono- or di- alkylamino, alkyl or aryl or heteroaryl amide, NCOR^{22} , $\text{NR}^{20}\text{SO}_2\text{R}^{22}$, COR^{20} , CO_2R^{20} , $\text{CON}(\text{R}^{20})_2$, $\text{NR}^{20}\text{CON}(\text{R}^{20})_2$, $\text{OC}(\text{O})\text{R}^{20}$, $\text{OC}(\text{O})\text{N}(\text{R}^{20})_2$, SR^{20} , $\text{S}(\text{O})\text{R}^{22}$, SO_2R^{22} , $\text{SO}_2\text{N}(\text{R}^{20})_2$, CN , or OR^{20} ;

R^5 and R^6 are each individually selected from H, and $\text{C}_1\text{-C}_{15}$ alkyl that is optionally substituted with from 1 to 2 substituents independently selected from the group of halo, NO_2 , heterocyclyl, aryl, heteroaryl, CF_3 , CN , OR^{20} , SR^{20} , $\text{N}(\text{R}^{20})_2$, $\text{S}(\text{O})\text{R}^{22}$, SO_2R^{22} , $\text{SO}_2\text{N}(\text{R}^{20})_2$, $\text{SO}_2\text{NR}^{20}\text{COR}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CON}(\text{R}^{20})_2$, $\text{N}(\text{R}^{20})_2\text{NR}^{20}\text{COR}^{22}$, $\text{NR}^{20}\text{CO}_2\text{R}^{22}$,

selected from halo, alkyl, mono- or dialkylamino, alkyl or aryl or heteroaryl amide, CN, O-C₁₋₆ alkyl, CF₃, aryl, and heteroaryl;

R²² is selected from the group consisting of C₁₋₁₅ alkyl, C₂₋₁₅ alkenyl, C₂₋₁₅ alkynyl, heterocyclyl, aryl, and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heterocyclyl, aryl, and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from halo, alkyl, mono- or dialkylamino, alkyl or aryl or heteroaryl amide, CN, O-C₁₋₆ alkyl, CF₃, aryl, and heteroaryl; and

wherein R² and R⁴ are selected from the group consisting of H, C₁₋₆ alkyl and aryl, wherein the alkyl and aryl substituents are optionally substituted with halo, CN, CF₃, OR²⁰ and N(R²⁰)₂ with the proviso that when R² is not hydrogen then R⁴ is hydrogen, and when R⁴ is not hydrogen then R² is hydrogen.

In preferred compounds of this invention, R³ is selected from the group consisting of C₁₋₁₅ alkyl, halo, CF₃, CN, OR²⁰, SR²⁰, S(O)R²², SO₂R²², SO₂N(R²⁰)₂, COR²⁰, CO₂R²⁰, -CONR⁷R⁸, aryl and heteroaryl wherein the alkyl, aryl and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, aryl, heteroaryl, CF₃, CN, OR²⁰, SR²⁰, S(O)R²², SO₂R²², SO₂N(R²⁰)₂, COR²⁰, CO₂R²⁰ or CON(R²⁰)₂, and each optional heteroaryl and aryl substituent is optionally substituted with halo, alkyl, CF₃, CN, and OR²⁰; R⁵ and R⁶ are independently selected from the group of H and C₁₋₁₅ alkyl including one optional aryl substituent and each optional aryl substituent that is optionally substituted with halo or CF₃; R⁷ is selected from the group consisting of C₁₋₁₅ alkyl, C₂₋₁₅ alkynyl, aryl, and heteroaryl, wherein the alkyl, alkynyl, aryl, and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, aryl, heteroaryl, CF₃, CN, OR²⁰, and each optional heteroaryl and aryl substituent is optionally substituted with halo, alkyl, CF₃, CN, or OR²⁰; R⁸ is selected from the group consisting of hydrogen and C₁₋₁₅ alkyl; R²⁰ is selected from the group consisting of H, C₁₋₄ alkyl and aryl, wherein alkyl and aryl substituents are optionally substituted with one alkyl substituent; and R²² is selected from the group consisting of C₁₋₄ alkyl and aryl which are each optionally substituted with from 1 to 3 alkyl group.

In more preferred compounds, R¹ is CH₂OH; R³ is selected from the group consisting of CO₂R²⁰, -CONR⁷R⁸ and aryl where the aryl substituent is optionally substituted with from 1 to 2 substituents independently selected from the group consisting of halo, C₁₋₆ alkyl, CF₃, and OR²⁰; R⁷ is selected from the group consisting of hydrogen, C₁₋₈ alkyl and aryl, where the alkyl and aryl substituents are optionally substituted with one substituent selected from the

group consisting of halo, aryl, CF_3 , CN, OR^{20} and wherein each optional aryl substituent is optionally substituted with halo, alkyl, CF_3 , CN, and OR^{20} ; R^8 is selected from the group consisting of hydrogen and C_{1-8} alkyl; and R^{20} is selected from hydrogen and C_{1-4} alkyl.

5 In a still more preferred embodiment, $\text{R}^1 = \text{CH}_2\text{OH}$; R^3 is selected from the group consisting of CO_2R^{20} , $-\text{CONR}^7\text{R}^8$, and aryl that is optionally substituted with one substituent selected from the group consisting of halo, C_{1-3} alkyl and OR^{20} ; R^7 is selected from of hydrogen, and C_{1-3} alkyl; R^8 is hydrogen; and R^{20} is selected from hydrogen and C_{1-4} alkyl. In this preferred embodiment, R^3 is most preferably selected from $-\text{CO}_2\text{Et}$ and $-\text{CONHEt}$.

10 In another still more preferred embodiment, $\text{R}^1 = -\text{CONHEt}$, R^3 is selected from the group consisting of CO_2R^{20} , $-\text{CONR}^7\text{R}^8$, and aryl in that aryl is optionally substituted with from 1 to 2 substituents independently selected from the group consisting of halo, C_{1-3} alkyl, CF_3 , or OR^{20} ; R^7 is selected from the group consisting of hydrogen, and C_{1-8} alkyl that is optionally substituted with one substituent selected from the group consisting of halo, CF_3 , CN or OR^{20} ; R^8 is selected from the group consisting of hydrogen and C_{1-3} alkyl; and R^{20} is
15 selected from the group consisting of hydrogen and C_{1-4} alkyl. In this more preferred embodiment, R^8 is preferably hydrogen, R^7 is preferably selected from the group consisting of hydrogen, and C_{1-3} , and R^{20} is preferably selected from the group consisting of hydrogen and C_{1-4} alkyl.

In a most preferred embodiment, the compound of this invention is selected from
20 ethyl 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate, (4S,2R,3R,5R)-2-{6-amino-2-[4-(4-chlorophenyl)pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3,4-diol, (4S,2R,3R,5R)-2-{6-amino-2-[4-(4-methoxyphenyl)pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3,4-diol, (4S,2R,3R,5R)-2-{6-amino-2-[4-(4-methylphenyl)pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3,4-diol,
25 (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide, 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylic acid, (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N,N-dimethylcarboxamide, (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-ethylcarboxamide, 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxamide, 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-(cyclopentylmethyl)carboxamide, (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-[(4-

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with the exception of cyclopropyl, or within a straight chain or branched portion. Examples of alkenyl groups include ethenyl, propenyl, isopropenyl, butenyl, cyclohexenyl, cyclohexenylalkyl and the like. A substituted alkenyl is the straight chain alkenyl, branched alkenyl or cycloalkenyl group defined previously, independently substituted with 1 to 3 groups or substituents of halo, hydroxy, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, acyloxy, aryloxy, heteroaryloxy, amino optionally mono- or di-substituted with alkyl, aryl or heteroaryl groups, amidino, urea optionally substituted with alkyl, aryl, heteroaryl or heterocyclyl groups, aminosulfonyl optionally N-mono- or N,N-di-substituted with alkyl, aryl or heteroaryl groups, alkylsulfonylamino, arylsulfonylamino, heteroarylsulfonylamino, alkylcarbonylamino, arylcarbonylamino, heteroarylcarbonylamino, carboxy, alkoxycarbonyl, aryloxy, heteroaryloxy, or the like attached at any available point to produce a stable compound.

"Alkynyl" - alone or in combination means a straight or branched hydrocarbon containing 2-20, preferably 2-17, more preferably 2-10, even more preferably 2-8, most preferably 2-4, carbon atoms containing at least one, preferably one, carbon to carbon triple bond. Examples of alkynyl groups include ethynyl, propynyl, butynyl and the like. A substituted alkynyl refers to the straight chain alkynyl or branched alkenyl defined previously, independently substituted with 1 to 3 groups or substituents of halo, hydroxy, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, acyloxy, aryloxy, heteroaryloxy, amino optionally mono- or di-substituted with alkyl, aryl or heteroaryl groups, amidino, urea optionally substituted with alkyl, aryl, heteroaryl or heterocyclyl groups, aminosulfonyl optionally N-mono- or N,N-di-substituted with alkyl, aryl or heteroaryl groups, alkylsulfonylamino, arylsulfonylamino, heteroarylsulfonylamino, alkylcarbonylamino, arylcarbonylamino, heteroarylcarbonylamino, or the like attached at any available point to produce a stable compound.

"Alkyl alkenyl" refers to a group $-R-CR'=CR''R'''$, where R is lower alkyl, or substituted lower alkyl, R', R'', R''' may independently be hydrogen, halogen, lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, hetaryl, or substituted hetaryl as defined below.

"Alkyl alkynyl" refers to a groups $-RC\equiv CR'$ where R is lower alkyl or substituted lower alkyl, R' is hydrogen, lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, hetaryl, or substituted hetaryl as defined below.

"Alkoxy" denotes the group $-OR$, where R is lower alkyl, substituted lower alkyl, acyl,

aryl, substituted aryl, aralkyl, substituted aralkyl, heteroalkyl, heteroarylalkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, or substituted cycloheteroalkyl as defined.

"Alkylthio" denotes the group $-SR$, $-S(O)_{n=1-2}-R$, where R is lower alkyl, substituted lower alkyl, aryl, substituted aryl, aralkyl or substituted aralkyl as defined herein.

5 "Acyl" denotes groups $-C(O)R$, where R is hydrogen, lower alkyl substituted lower alkyl, aryl, substituted aryl and the like as defined herein.

"Aryloxy" denotes groups $-OAr$, where Ar is an aryl, substituted aryl, heteroaryl, or substituted heteroaryl group as defined herein.

10 "Amino" denotes the group NRR' , where R and R' may independently be hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, or substituted hetaryl as defined herein or acyl.

"Amido" denotes the group $-C(O)NRR'$, where R and R' may independently be hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, substituted hetaryl as defined herein.

15 "Carboxyl" denotes the group $-C(O)OR$, where R is hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, and substituted hetaryl as defined herein.

"Aryl" - alone or in combination means phenyl or naphthyl optionally carbocyclic fused with a cycloalkyl of preferably 5-7, more preferably 5-6, ring members and/or optionally substituted with 1 to 3 groups or substituents of halo, hydroxy, alkoxy, alkylthio, 20 alkylsulfinyl, alkylsulfonyl, acyloxy, aryloxy, heteroaryloxy, amino optionally mono- or di-substituted with alkyl, aryl or heteroaryl groups, amidino, urea optionally substituted with alkyl, aryl, heteroaryl or heterocyclyl groups, aminosulfonyl optionally N-mono- or N,N-di-substituted with alkyl, aryl or heteroaryl groups, alkylsulfonylamino, arylsulfonylamino, heteroarylsulfonylamino, alkylcarbonylamino, arylcarbonylamino, heteroarylcarbonylamino, 25 or the like.

"Substituted aryl" refers to aryl optionally substituted with one or more functional groups, e.g., halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

30 "Heterocycle" refers to a saturated, unsaturated, or aromatic carbocyclic group having a single ring (e.g., morpholino, pyridyl or furyl) or multiple condensed rings (e.g., naphthpyridyl, quinoxalyl, quinolinyl, indolizinyll or benzo[b]thienyl) and having at least one hetero atom, such as N, O or S, within the ring, which can optionally be unsubstituted or substituted with, e.g., halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido,

carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

“Heteroaryl” - alone or in combination means a monocyclic aromatic ring structure containing 5 or 6 ring atoms, or a bicyclic aromatic group having 8 to 10 atoms, containing one or more, preferably 1-4, more preferably 1-3, even more preferably 1-2, heteroatoms independently selected from the group O, S, and N, and optionally substituted with 1 to 3 groups or substituents of halo, hydroxy, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, acyloxy, aryloxy, heteroaryloxy, amino optionally mono- or di-substituted with alkyl, aryl or heteroaryl groups, amidino, urea optionally substituted with alkyl, aryl, heteroaryl or heterocyclyl groups, aminosulfonyl optionally N-mono- or N,N-di-substituted with alkyl, aryl or heteroaryl groups, alkylsulfonylamino, arylsulfonylamino, heteroarylsulfonylamino, alkylcarbonylamino, arylcarbonylamino, heteroarylcarbonylamino, or the like. Heteroaryl is also intended to include oxidized S or N, such as sulfinyl, sulfonyl and N-oxide of a tertiary ring nitrogen. A carbon or nitrogen atom is the point of attachment of the heteroaryl ring structure such that a stable aromatic ring is retained. Examples of heteroaryl groups are pyridinyl, pyridazinyl, pyrazinyl, quinazolinyl, purinyl, indolyl, quinolinyl, pyrimidinyl, pyrrolyl, oxazolyl, thiazolyl, thienyl, isoxazolyl, oxathiadiazolyl, isothiazolyl, tetrazolyl, imidazolyl, triazinyl, furanyl, benzofuryl, indolyl and the like. A substituted heteroaryl contains a substituent attached at an available carbon or nitrogen to produce a stable compound.

“Heterocyclyl” - alone or in combination means a non-aromatic cycloalkyl group having from 5 to 10 atoms in which from 1 to 3 carbon atoms in the ring are replaced by heteroatoms of O, S or N, and are optionally benzo fused or fused heteroaryl of 5-6 ring members and/or are optionally substituted as in the case of cycloalkyl. Heterocyclyl is also intended to include oxidized S or N, such as sulfinyl, sulfonyl and N-oxide of a tertiary ring nitrogen. The point of attachment is at a carbon or nitrogen atom. Examples of heterocyclyl groups are tetrahydrofuranyl, dihydropyridinyl, piperidinyl, pyrrolidinyl, piperazinyl, dihydrobenzofuryl, dihydroindolyl, and the like. A substituted heterocyclyl contains a substituent nitrogen attached at an available carbon or nitrogen to produce a stable compound.

“Substituted heteroaryl” refers to a heterocycle optionally mono or poly substituted with one or more functional groups, *e.g.*, halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

“Aralkyl” refers to the group -R-Ar where Ar is an aryl group and R is lower alkyl or

substituted lower alkyl group. Aryl groups can optionally be unsubstituted or substituted with, *e.g.*, halogen, lower alkyl, alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

5 "Heteroalkyl" refers to the group -R-Het where Het is a heterocycle group and R is a lower alkyl group. Heteroalkyl groups can optionally be unsubstituted or substituted with *e.g.*, halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

10 "Heteroarylalkyl" refers to the group -R-HetAr where HetAr is an heteroaryl group and R lower alkyl or substituted lower alkyl. Heteroarylalkyl groups can optionally be unsubstituted or substituted with, *e.g.*, halogen, lower alkyl, substituted lower alkyl, alkoxy, alkylthio, acetylene, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

15 "Cycloalkyl" refers to a divalent cyclic or polycyclic alkyl group containing 3 to 15 carbon atoms.

 "Substituted cycloalkyl" refers to a cycloalkyl group comprising one or more substituents with, *e.g.*, halogen, lower alkyl, substituted lower alkyl, alkoxy, alkylthio, acetylene, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl,
20 nitro, cyano, thiol, sulfamido and the like.

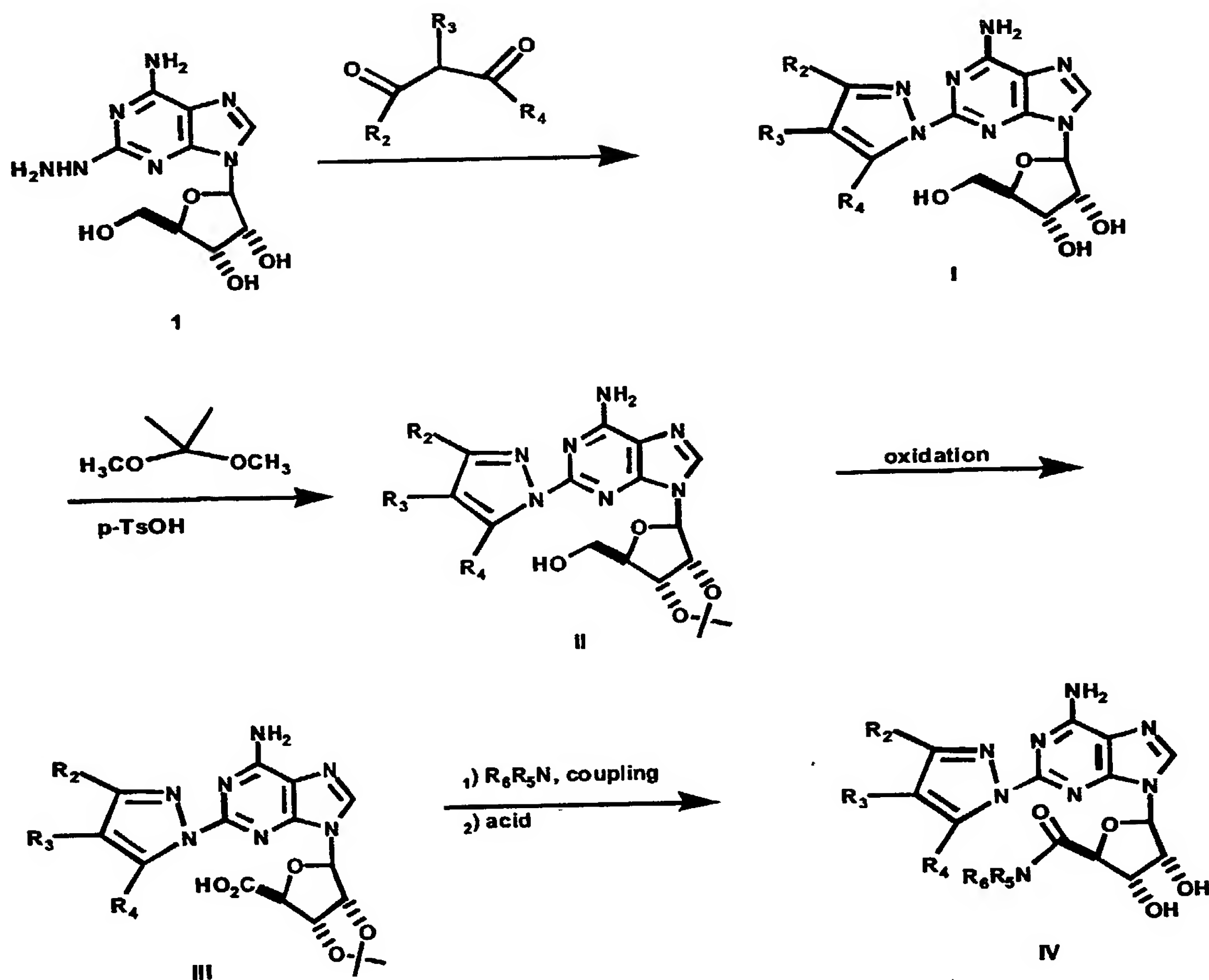
 "Cycloheteroalkyl" refers to a cycloalkyl group wherein one or more of the ring carbon atoms is replaced with a heteroatom (*e.g.*, N, O, S or P).

 Substituted cycloheteroalkyl" refers to a cycloheteroalkyl group as herein defined which contains one or more substituents, such as halogen, lower alkyl, lower alkoxy,
25 alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

 "Alkyl cycloalkyl" denotes the group -R-cycloalkyl where cycloalkyl is a cycloalkyl group and R is a lower alkyl or substituted lower alkyl. Cycloalkyl groups can optionally be unsubstituted or substituted with *e.g.* halogen, lower alkyl, lower alkoxy, alkylthio, acetylene,
30 amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

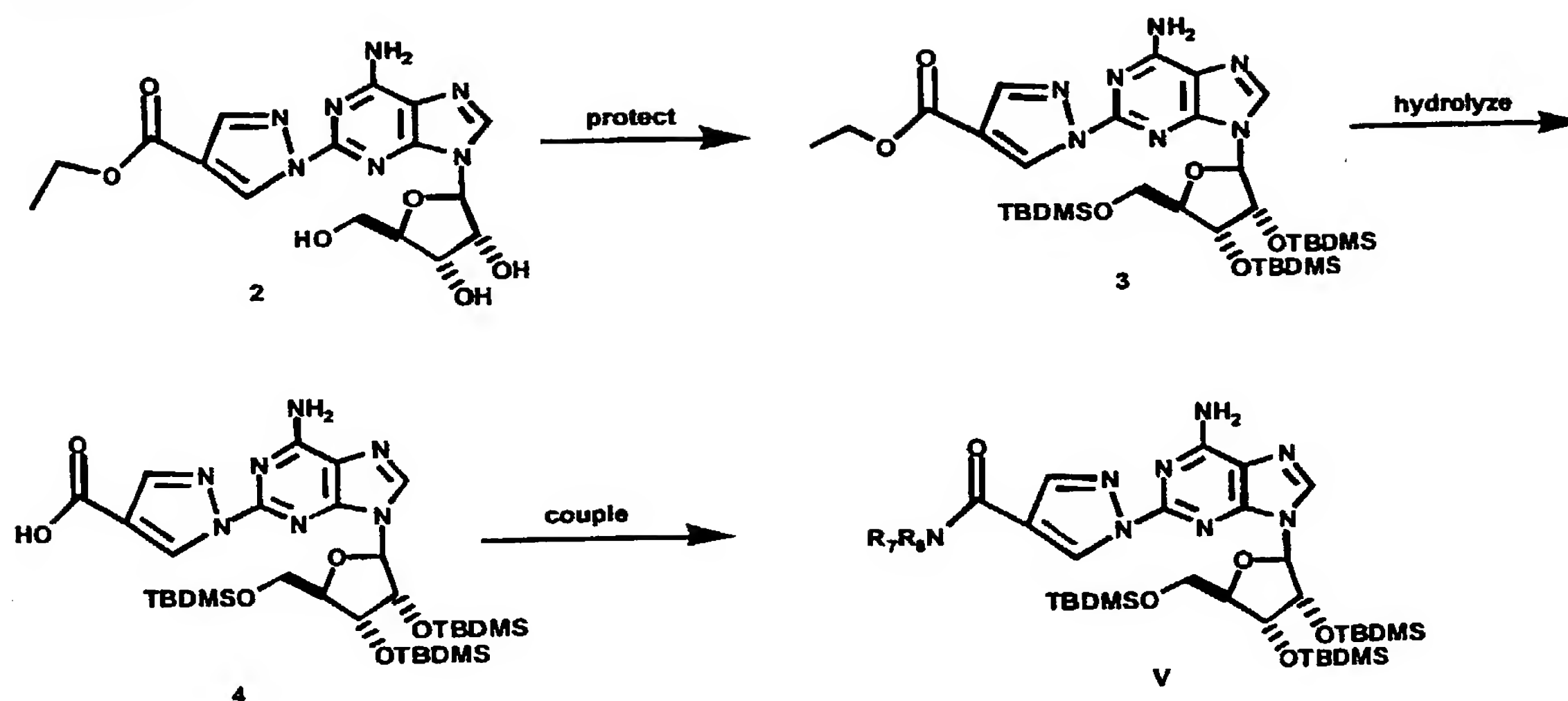
 "Alkyl cycloheteroalkyl" denotes the group -R-cycloheteroalkyl where R is a lower alkyl or substituted lower alkyl. Cycloheteroalkyl groups can optionally be unsubstituted or substituted with *e.g.* halogen, lower alkyl, lower alkoxy, alkylthio, amino, amido, carboxyl,

Scheme 1.



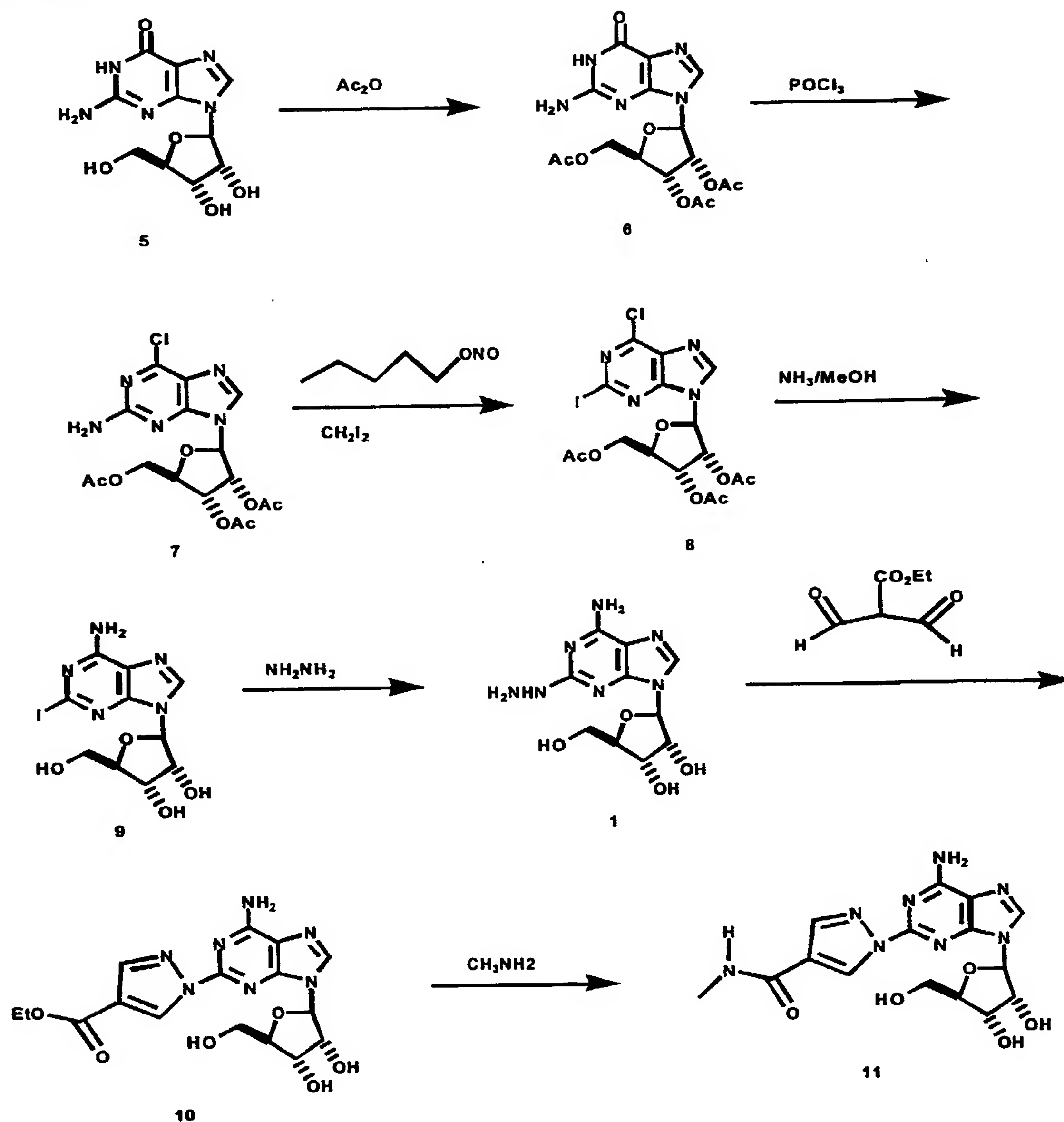
Compound I can be prepared by reacting compound 1 with appropriately substituted 1,3-dicarbonyl in a mixture of AcOH and MeOH at 80°C (Holzer et al., J. Heterocycl. Chem. (1993) 30, 865). Compound II, which can be obtained by reacting compound I with 2,2-dimethoxypropane in the presence of an acid, can be oxidized to the carboxylic acid III, based on structurally similar compounds using potassium permanganate or pyridinium chlorochromate (M. Hudlicky, (1990) Oxidations in Organic Chemistry, ACS Monographs, American Chemical Society, Washington D. C.). Reaction of a primary or secondary amine having the formula HNR⁶R⁷, and compound III using DCC (M. Fujino et al., Chem. Pharm. Bull. (1974), 22, 1857), PyBOP (J. Martinez et al., J. Med. Chem. (1988) 28, 1874) or PyBrop (J. Caste et al. Tetrahedron, (1991), 32, 1967) coupling conditions can afford compound IV.

Scheme 2.



Compound V can be prepared as shown in Scheme 2. The Tri TBDMS derivative 4 can be obtained by treating compound 2 with TBDMSCl and imidazole in DMF followed by hydrolysis of the ethyl ester using NaOH. Reaction of a primary or secondary amine with the formula HNR⁶R⁷, and compound 4 using DCC (M. Fujino et al., Chem. Pharm. Bull. (1974), 22, 1857), PyBOP (J. Martinez et al., J. Med. Chem. (1988) 28, 1874) or PyBrop (J. Caste et al. Tetrahedron, (1991), 32, 1967) coupling conditions can afford compound V.

Scheme 3



A specific synthesis of Compound 11 is illustrated in Scheme 3. Commercially available guanosine 5 was converted to the triacetate 6 as previously described (M. J. Robins and B. Uznanski, *Can. J. Chem.* (1981), 59, 2601-2607). Compound 7, prepared by following the literature procedure of Cerster et al. (J. F. Cerster, A. F. Lewis, and R.K. Robins, *Org. Synthesis*, 242-243), was converted to compound 9 in two steps as previously described (V. Nair et al., *J. Org. Chem.*, (1988), 53, 3051-3057). Compound 1 was obtained by reacting hydrazine hydrate with compound 9 in ethanol at 80°C . Condensation of compound 1 with

through their anti-inflammatory action on neutrophils. Thus, in situations when the heart will go through an ischemic state such as a transplant, they will be useful.

This invention also includes pro-drugs of the above-identified A_{2A} agonists. A pro-drug is a drug which has been chemically modified and may be biological inactive at its site of action, but which will be degraded or modified by one or more enzymatic or *in vivo* processes to the bioactive form. The pro-drugs of this invention should have a different pharmacokinetic profile to the parent enabling improved absorption across the mucosal epithelium, better salt formulation and/or solubility and improved systemic stability. The above-identified compounds may be preferably modified at one or more of the hydroxyl groups. The modifications may be (1) ester or carbamate derivatives which may be cleaved by esterases or lipases, for example; (2) peptides which may be recognized by specific or non specific proteinase; or (3) derivatives that accumulate at a site of action through membrane selection or a pro-drug form or modified pro-drug form, or any combination of (1) to (3) above.

The compounds may be administered orally, intravenously, through the epidermis or by any other means known in the art for administering a therapeutic agents. The method of treatment comprises the administration of an effective quantity of the chosen compound, preferably dispersed in a pharmaceutical carrier. Dosage units of the active ingredient are generally selected from the range of 0.01 to 100 mg/kg, but will be readily determined by one skilled in the art depending upon the route of administration, age and condition of the patient. This dose is typically administered in a solution about 5 minutes to about an hour or more prior to coronary imaging. No unacceptable toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

If the final compound of this invention contains a basic group, an acid addition salt may be prepared. Acid addition salts of the compounds are prepared in a standard manner in a suitable solvent from the parent compound and an excess of acid, such as hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, maleic, succinic, or methanesulfonic. The hydrochloric salt form is especially useful. If the final compound contains an acidic group, cationic salts may be prepared. Typically the parent compound is treated with an excess of an alkaline reagent, such as hydroxide, carbonate or alkoxide, containing the appropriate cation. Cations such as Na^+ , K^+ , Ca^{+2} and NH_4^+ are examples of cations present in pharmaceutically acceptable salts. Certain of the compounds form inner salts or zwitterions which may also be acceptable.

Pharmaceutical compositions including the compounds of this invention, and/or

derivatives thereof, may be formulated as solutions or lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. If used in liquid form the compositions of this invention are preferably incorporated into a buffered, isotonic, aqueous solution.

5 Examples of suitable diluents are normal isotonic saline solution, standard 5% dextrose in water and buffered sodium or ammonium acetate solution. Such liquid formulations are suitable for parenteral administration, but may also be used for oral administration. It may be desirable to add excipients such as polyvinylpyrrolidinone, gelatin, hydroxycellulose, acacia, polyethylene glycol, mannitol, sodium chloride, sodium citrate or any other excipient

10 known to one of skill in the art to pharmaceutical compositions including compounds of this invention. Alternatively, the pharmaceutical compounds may be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Liquid carriers include syrup, peanut oil, olive oil, glycerin,

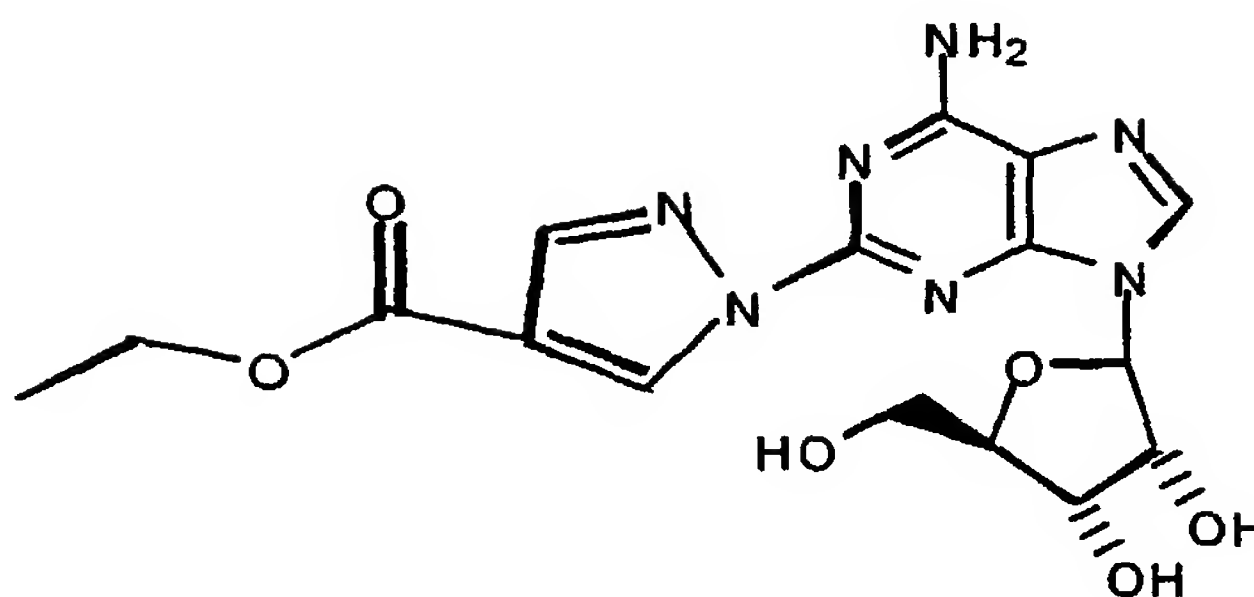
15 saline, alcohols and water. Solid carriers include starch, lactose, calcium sulfate, dihydrate, teffa alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. The carrier may also include a sustained release material such as glycerol monostearate or glycerol distearate, alone or with a wax. The amount of solid carrier varies but, preferably, will be between about 20 mg to about 1 gram per dosage unit. The pharmaceutical dosages are

20 made using conventional techniques such as milling, mixing, granulation, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly or filled into a soft gelatin capsule. It is preferred that the

25 compositions of this invention are administered as a solution either orally or intravenously by continuous infusion or bolus.

The Examples which follow serve to illustrate this invention. The Examples are intended to in no way limit the scope of this invention, but are provided to show how to make and use the compounds of this invention. In the Examples, all temperatures

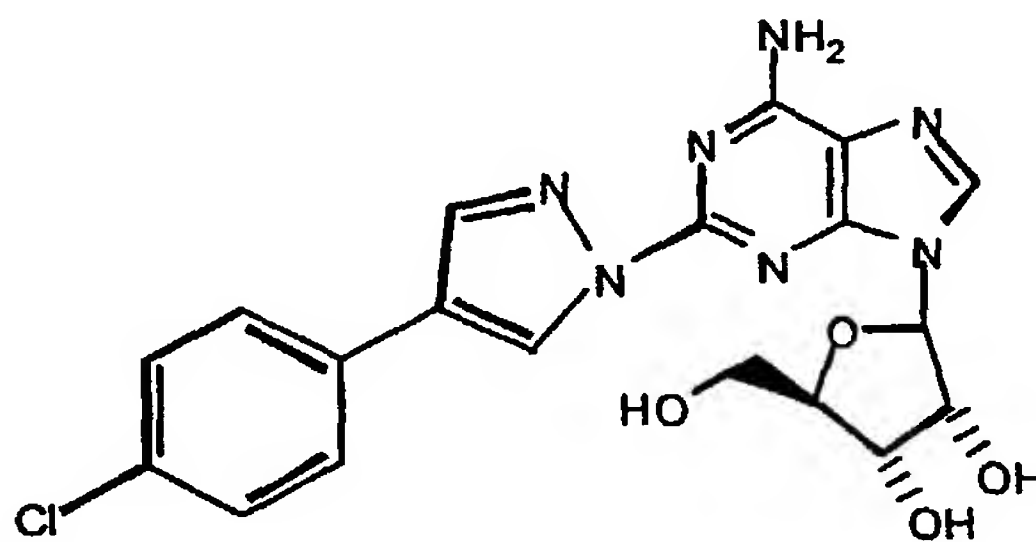
30 are in degrees Centigrade.

Example 1

ethyl 1-(9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl)pyrazole-4-carboxylate (12)

5 To a suspension of 2-hydrazinoadenosine (0.025 g, 0.08 mmol) in a 1:1 mixture of MeOH/AcOH was added (ethoxycarbonyl)malondialdehyde (0.019 g, 0.12 mmol) and the mixture was heated at 80°C for 3 h. The precipitate formed was collected by filtration and washed with EtOH and ether to afford 12. ¹HNMR (DMSO-d₆) δ 1.25 (t, 3 H), 3.5 (m, 1 H), 3.6 (m, 1 H), 3.8 (d, 1 H), 4.15 (d, 1 H), 4.55 (m, 1H), 5.0 (t, 1 H), 5.2 (d, 1 H), 5.5 (d, 1 H), 5.9 (d, 1H), 7.15-7.3 (m, 5 H), 7.8 (br s, 2 H), 8.1 (s, 1H), 8.4 (s, 1 H), 8.9 (s, 1H).

10

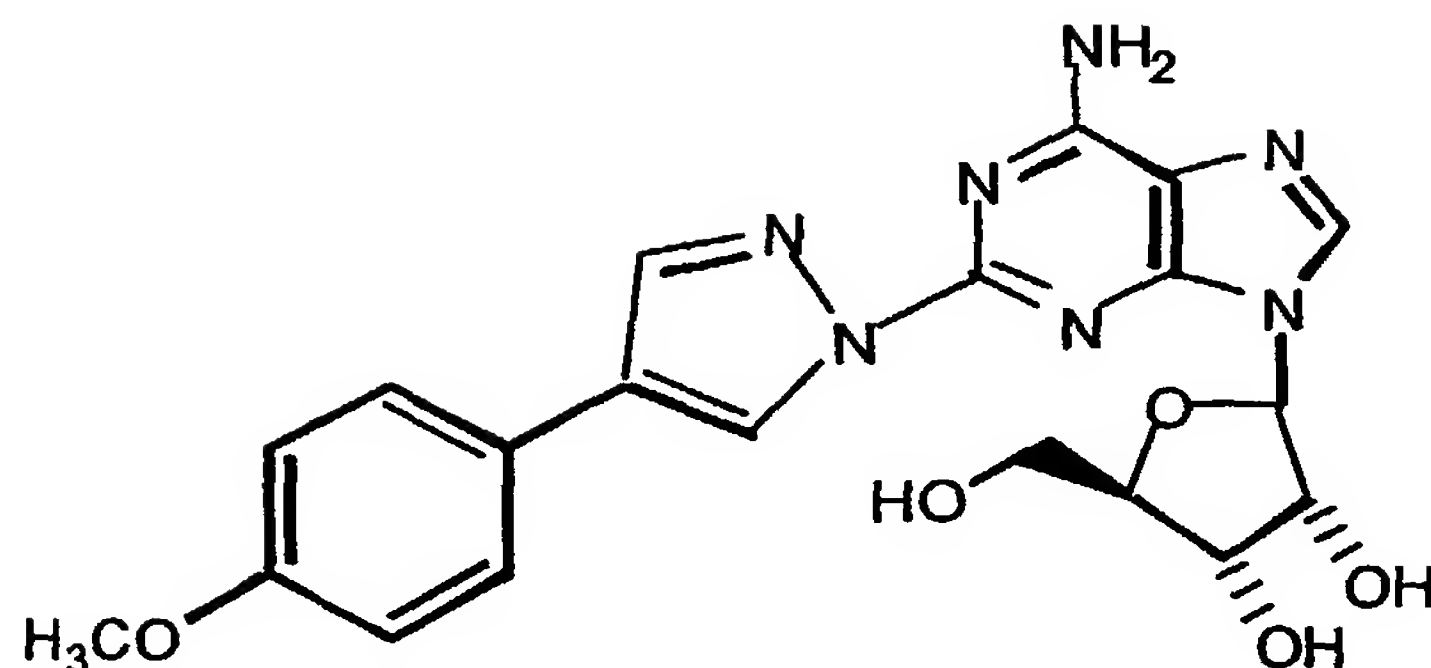
Example 2

(4S,2R,3R,5R)-2-{6-amino-2-[4-(4-chlorophenyl)pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3,4-diol (13)

15

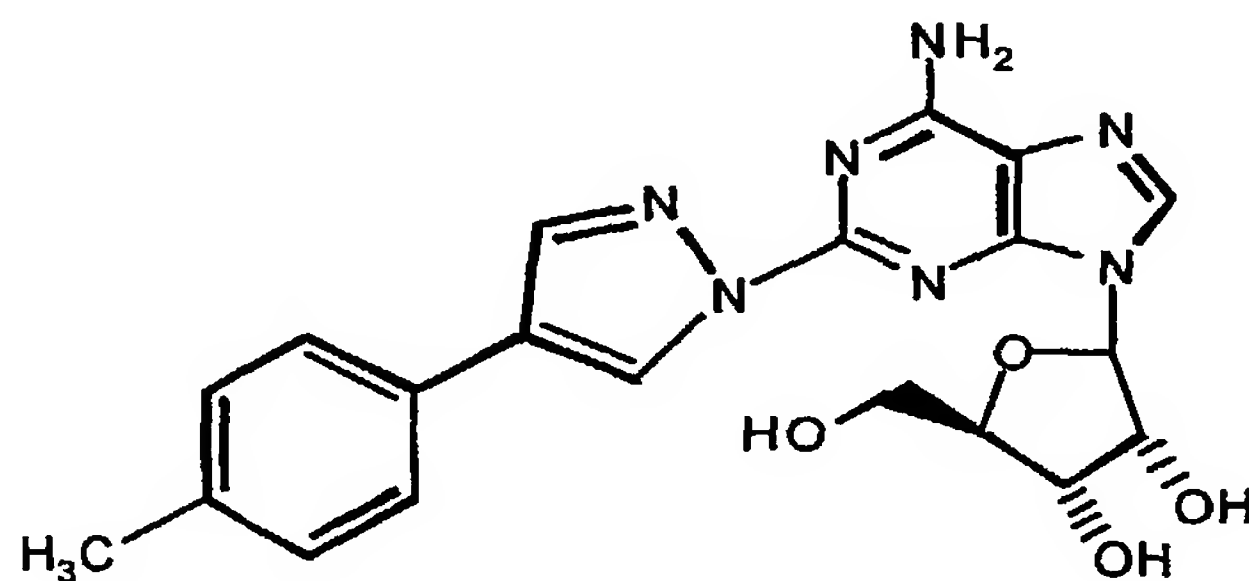
To a suspension of 2-hydrazinoadenosine (0.025 g, 0.08 mmol) in a 1:1 mixture of MeOH/AcOH was added 2-(4-chloro)phenylmalondialdehyde (0.022g, 0.12 mmol) and the mixture was heated at 80 °C for 3 h. The precipitate formed was collected by filtration and washed with EtOH and Ether to afford 13. ¹HNMR (DMSO-d₆) δ 3.5 (m, 1 H), 3.6 (m, 1 H), 3.8 (d, 1 H), 4.15 (d, 1 H), 4.2 (q, 2 H), 4.55 (m, 1H), 5.9 (d, 1H), 7.45 (d, 2 H), 7.75 (d, 2 H), 8.25 (s, 1H), 8.35 (s, 1 H), 8.9 (s, 1H).

20

Example 3

5 **(4S,2R,3R,5R)-2-{6-amino-2-[4-(4-methoxyphenyl)pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3,4-diol (14)**

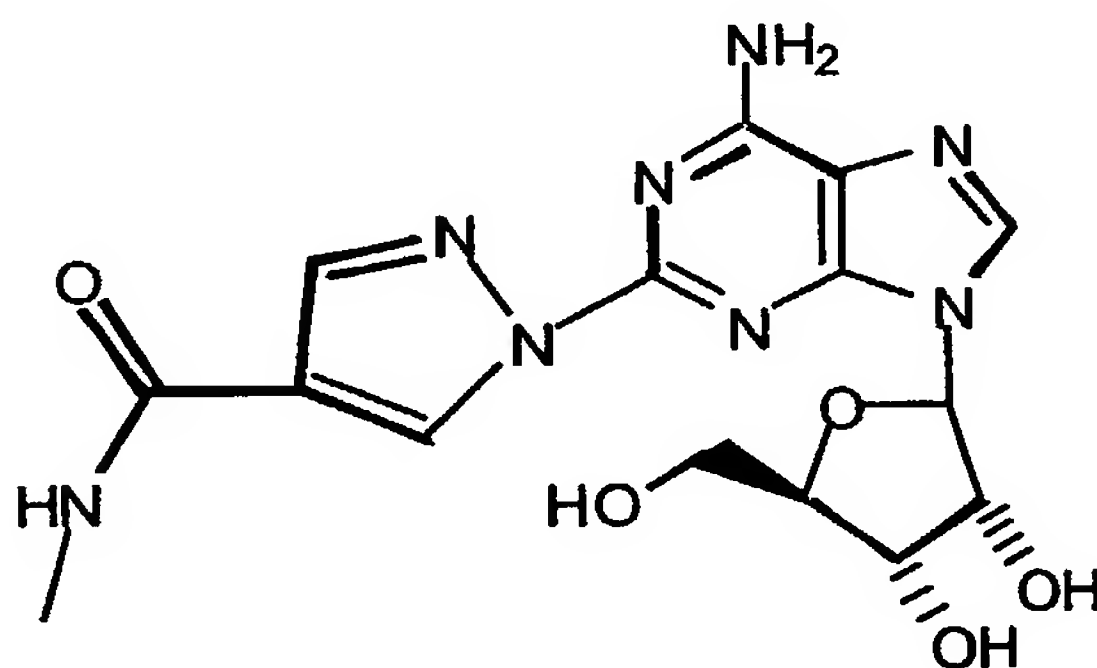
To a suspension of 2-hydrazinoadenosine (0.025 g, 0.08 mmol) in a 1:1 mixture of MeOH/AcOH was added 2-(4-methoxy)phenylmalondialdehyde (0.022g, 0.12 mmol) and the mixture was heated at 80 °C for 3 h. The precipitate formed was collected by filtration and
 10 washed with EtOH and Ether to afford 14. ¹HNMR (DMSO-d₆) δ3.55 (m, 1 H), 3.65 (m, 1 H), 3.75 (s, 3 H), 3.9 (d, 1 H), 4.15 (d, 1 H), 4.6 (m, 1 H), 5.9 (d, 1 H), 6.75 (d, 2 H), 7.6 (d, 2 H), 8.15 (s, 1H), 8.35 (s, 1 H), 8.8 (s, 1 H).

Example 4

20 **(4S,2R,3R,5R)-2-{6-amino-2-[4-(4-methylphenyl)pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3,4-diol (15)**

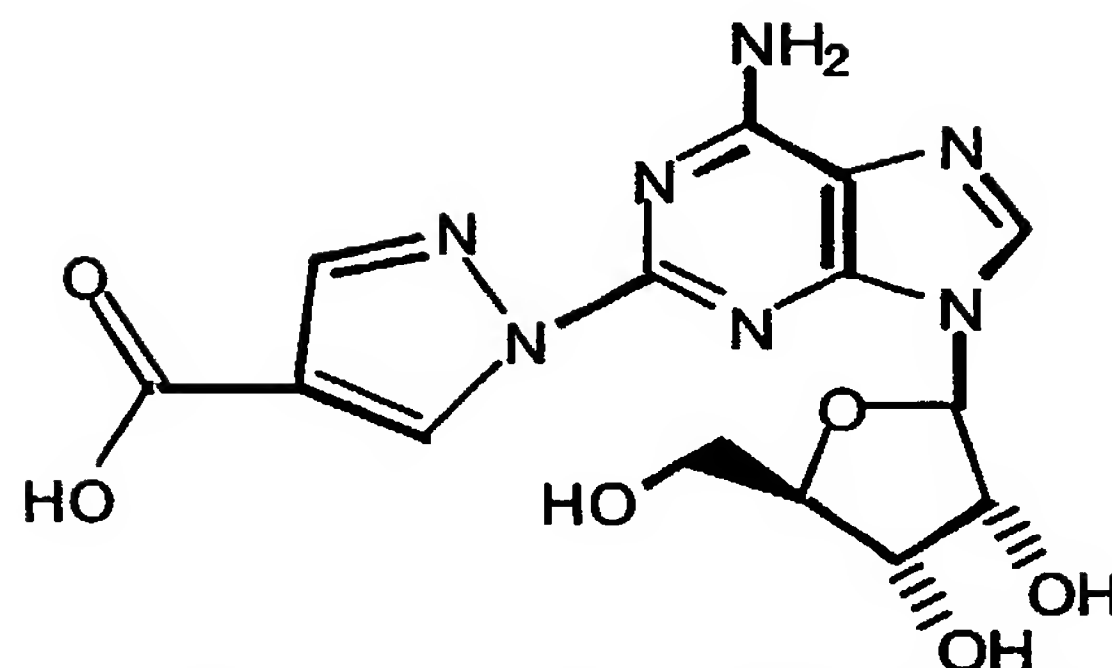
To a suspension of 2-hydrazinoadenosine (0.025 g, 0.08 mmol) in a 1:1 mixture of MeOH/AcOH was added 2-(4-methyl)phenylmalondialdehyde (0.019g, 0.12 mmol) and the mixture was heated at 80 °C for 3 h. The precipitate formed was collected by filtration and washed with EtOH and Ether to afford 15. ¹HNMR (DMSO-d₆) δ3.55 (m, 1 H), 3.65 (m, 1 H), 3.75 (s, 3 H), 3.9 (d, 1 H), 4.15 (d, 1 H), 4.6 (m, 1 H), 5.9 (d, 1 H), 6.75 (d, 2 H), 7.6 (d, 2 H), 8.15 (s, 1 H), 8.35 (s, 1 H), 8.8 (s, 1 H).

Example 5



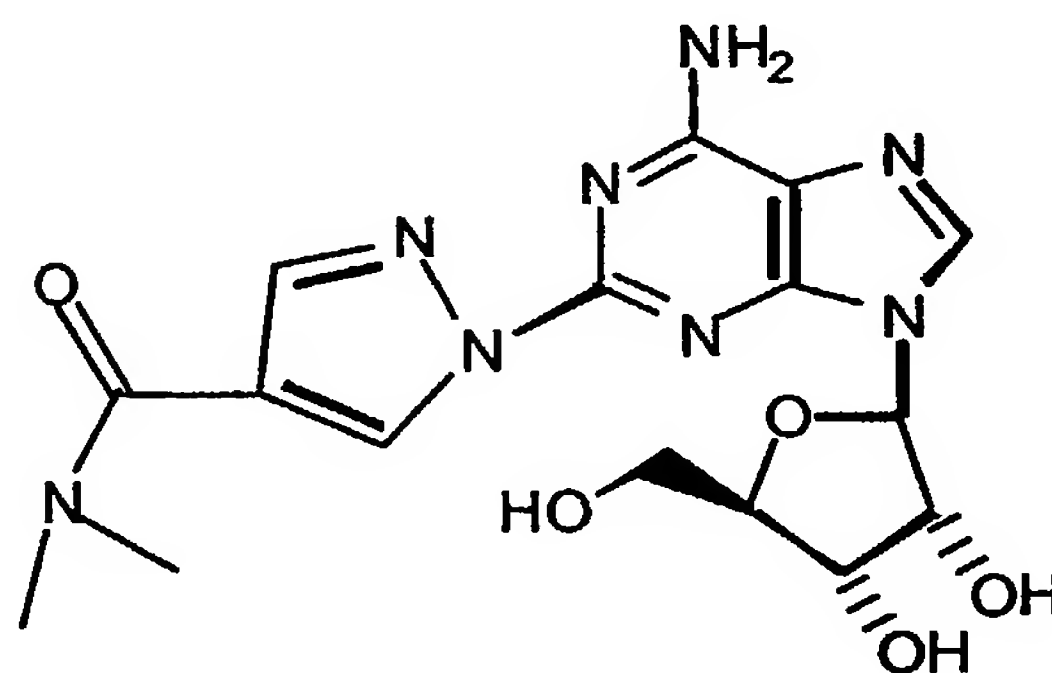
(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide (16)

Compound 12 (0.05 mg, 0.12 mmol) was added to 4 mL methylamine (40% sol. In water). The mixture heated at 65 °C in for 24 h. After concentration in vacuo, the residue was purified using prep. TLC (10% MeOH:DCM). ¹HNMR (CD₃OD) δ2.90 (s, 3 H), 3.78 (m, 1 H), 3.91 (m, 1 H), 4.13 (d, 1 H), 4.34 (d, 1 H), 4.64 (m, 1 H), 6.06 (d, 1 H), 8.11 (s, 1 H), 8.38 (s, 1 H), 9.05 (s, 1 H).

Example 6

1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylic acid (17)

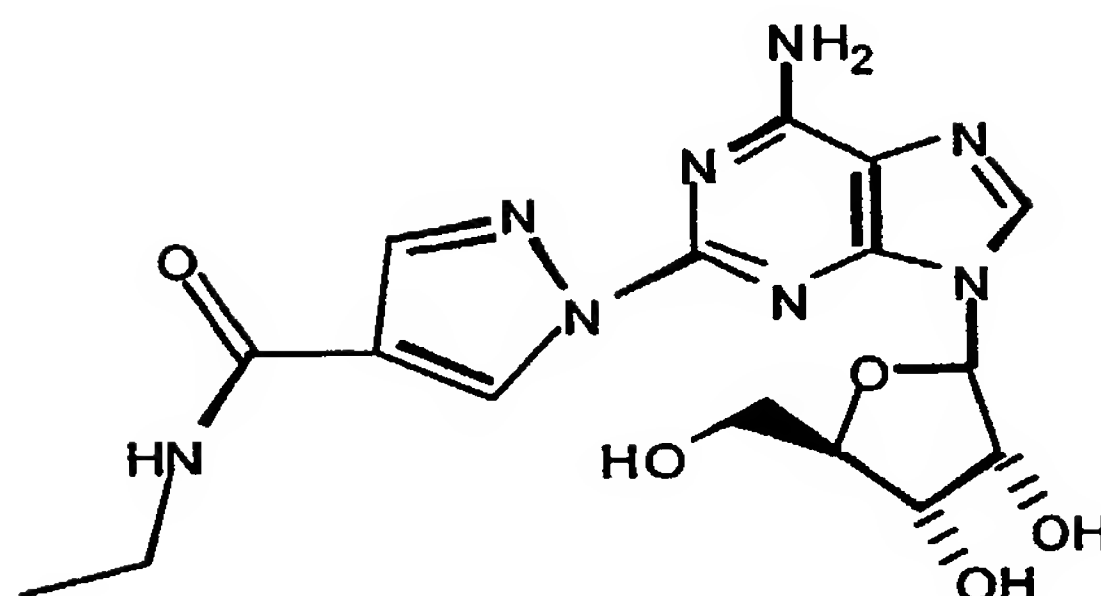
Compound 12 (0.05 mg, 0.12 mmol) was dissolved one equivalent of 1N NaOH. The solution was allowed to stir at Rt for 2h, then acidified to pH 4. The resulting precipitate was filtered and washed with water and ether. ¹HNMR (CD₃OD) Δ3.75 (m, 1 H), 3.90 (m, 1 H), 4.13 (d, 1 H), 4.43 (d, 1 H), 4.64 (m, 1H), 6.05 (d, 1H), 8.10 (s, 1H), 8.35 (s, 1 H), 9.05 (s, 1 H).

Example 7

(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N,N-dimethylcarboxamide (18)

Compound 18 was prepared in a manner similar to that of compound 16 using dimethylamine instead of methylamine, MS 405.12 (M+1).

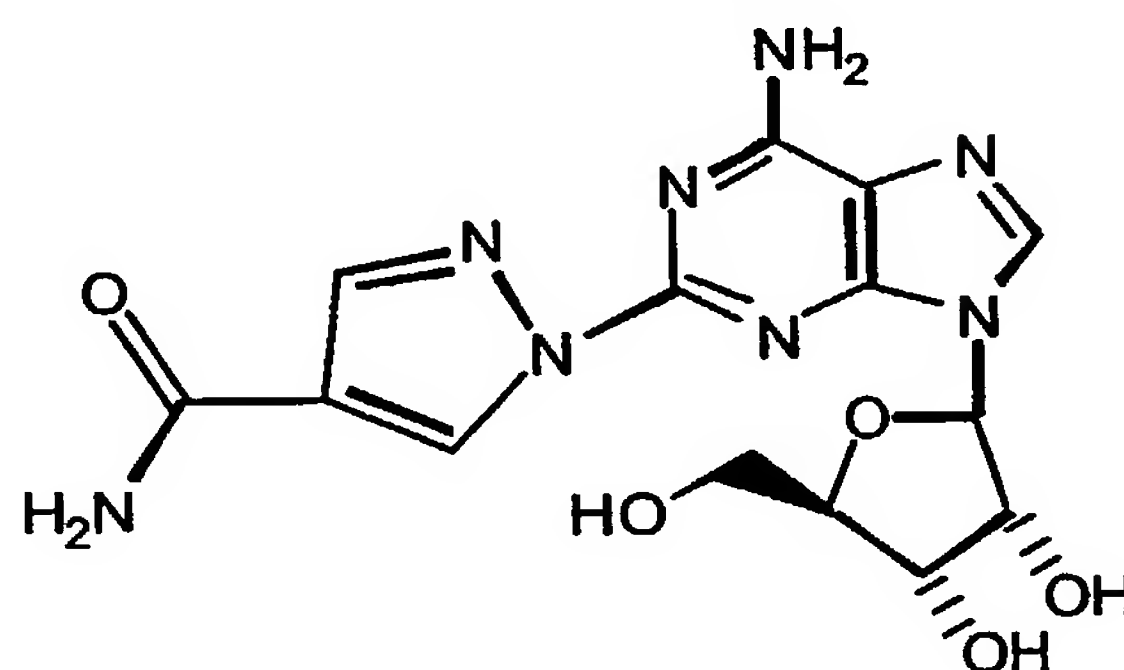
Example 8



(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-ethylcarboxamide (19)

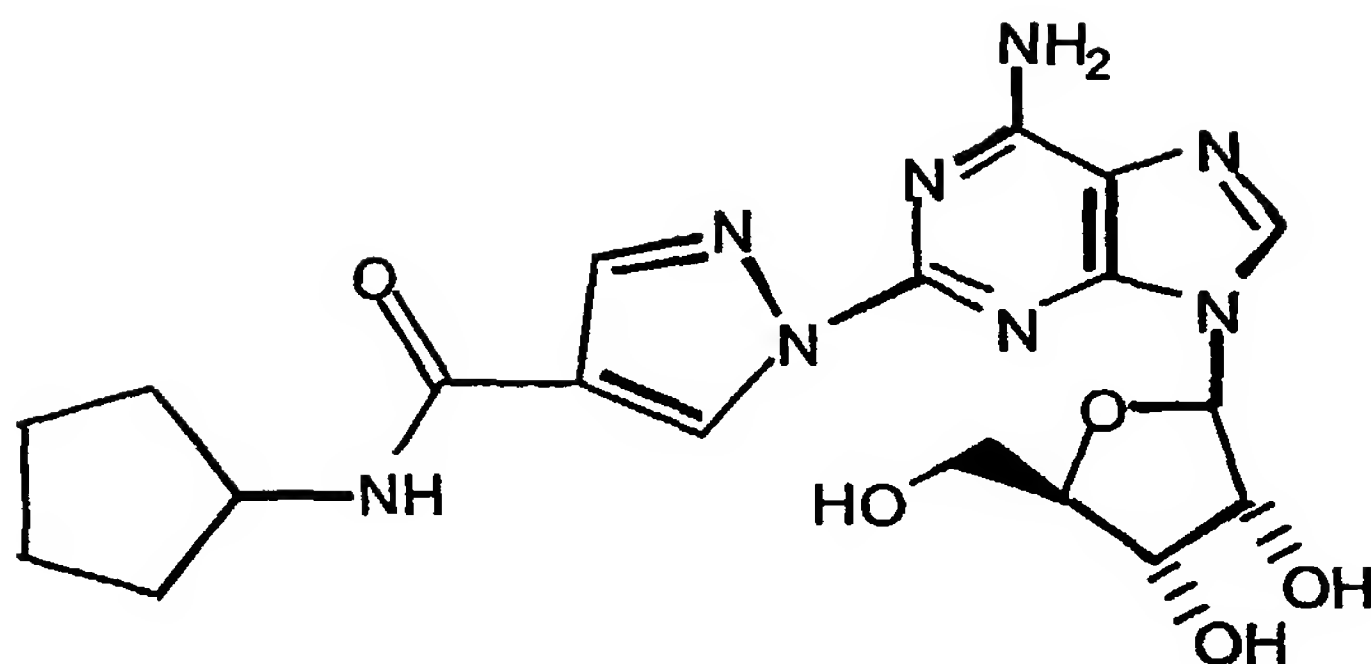
Compound 19 was prepared in a manner similar to that of compound 16 using ethylamine instead of methylamine, MS 405.35 (M+1).

Example 9



1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxamide (20)

Compound **20** was prepared in a manner similar to that of compound **16** using ammonia instead of methylamine, MS 377.25 (M+1).

Example 10

5 (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-(cyclopentylmethyl)carboxamide (21)

Compound 12 (0.5 g, 1.2 mmol) was dissolved in dry DMF, TBDMSCl (1.5 g, 10 mmol) and imidazole (0.68 g, 10 mmol) were added and the mixture was heated at 80 °C for 24 h. The solvent was evaporated and the residue was purified by flash column to obtain the
10 trisilyl protected form of compound 12. The trisilyl derivative (0.8 g) was then suspended in 1 mL of water and treated with 2 mL 1N KOH/MeOH. The mixture was stirred at RT for 72. The solvent was removed under reduced pressure and the residue was suspended in 5 mL of water and acidified to pH 5.5 with 1N HCl. The resulting precipitate was filtered and washed with water and ethyl ether to afford the trisilyl form of the acid 20.

15 The trisilyl derivative acid 20 (0.14 g, 0.2 mmol) was then dissolved in 5 mL dichloromethane. To the solution was added HBTU (0.19 g, 0.4 mmol), HOBt (0.076 g, 0.4 mmol), N-methylmorpholine (0.04 g, 0.4 mmol) and cat. DMAP. The mixture was allowed to stir at RT for 24 h. The mixture was then washed with 10% citric acid, saturated NaHCO₃, brine and dried over MgSO₄. The solvent was removed and the residue was treated with 5 mL
20 0.5N NH₄F/MeOH. The solution was heated at reflux for 24 h. The solvent was evaporated and the residue was purified by preparative TLC to afford compound 21, MS 445.26 (M+1).

Example 14

Compounds of this invention were assayed to determine their affinity for the A_{2A} receptor in a pig striatum membrane prep. Briefly, 0.2 mg of pig striatal membranes were treated with adenosine deaminase (2 U/ mL) and 50 mM Tris buffer (pH = 7.4) followed by mixing. To the pig membranes was added 2 µL of serially diluted DMSO stock solution of the compounds of this invention at concentrations ranging from 10 nM to 100 microM or the control received 2 microL of DMSO alone, then the antagonist ZM 241385 in Tris buffer (50 mM, pH of 7.4) was added to achieve a final concentration of 2 nM. After incubation at 23 °C for 2h, then the solutions were filtered using a membrane harvester using multiple washing of the membranes (3 x). The filter disks were counted in scintillation cocktail to determine the amount of displacement of tritiated ZM displaced by the compounds of this invention. Greater than a 5 point curve was used to generate Ki.'s. and the number of experiments is indicated in the column marked in Table 1 below.

Table 1

Compound Number	A _{2A} Ki, nM	n
12	+++	2
13	++	3
14	++	1
15	++	3
16	++	2
17	-	1
18	+++	3
19	+++	3
20	+++	3
21	+++	3
22	+++	3

+++ = 10-1,000 nM

++ = 1,000-10,000 nM

+ = greater than 10,000 nM

- = greater than 100,000 nM

EXAMPLE 15

The objective of this experiment was to determine the affinities and receptor binding selectivity of a compound of this invention for A_1 , A_{2A} , A_{2B} and A_3 adenosine receptors. Molecular cloning has identified and confirmed the existence of four subtypes of adenosine receptors (AdoRs), designated as A_1 , A_{2A} , A_{2B} and A_3 AdoRs (Linden, 1994). These AdoR subtypes have distinct anatomical distributions, pharmacological properties and physiological functions (Shryock and Belardinelli, 1997). A_1 and A_3 AdoRs couple to inhibitory G proteins (G_i/o) and decrease the activity of adenylyl cyclase, whereas A_{2A} and A_{2B} AdoRs increase intracellular cAMP content via coupling to stimulatory G proteins (Gs).

Ligands with high potency and tissue/organ selectivity for distinct adenosine receptor subtypes have therapeutic and diagnostic potentials for a variety of diseases (such as arrhythmia, ischemic heart diseases, asthma and Parkinson's disease) and are the focus of considerable research efforts by both academia and industry. Here we report the pharmacological and functional characterization of a series of novel adenosine analogues of this invention using mammalian cell lines expressing either endogenous AdoRs or recombinant human AdoRs.

Materials

Adenosine deaminase was purchased from Boehringer Mannheim Biochemicals Indianapolis, IN, U.S.A). [3 H]ZM241385 (Lot No. 1) was purchased from Tocris Cookson Ltd (Langford, Bristol, UK). [3 H]CPX (Lot No. 3329207) was from New England Nuclear (Boston, MA, USA). CGS21680 (Lot No. SW-3R-84 and 89H4607), NECA (Lot No. OXV-295E), R-PIA (Lot No. WY-V-23), Rolipram and HEK-h A_{2A} AR membranes were obtained from Sigma-RBI (Natick, MA). WRC-0470 was prepared as described in the literature (K. Niiya et al., J. Med. Chem. 35; 4557-4561 (1992); Compound 16 of this invention was synthesized as described above and prepared as a stock solution (10 mmol/L) in DMSO.

Cell culture and membrane preparation-PC12 cells were obtained from the American Type Culture Collection and grown in DMEM with 5% fetal bovine serum, 10% horse serum, 0.5 mmol/L L-glutamine, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 2.5 μ g/mL amphotericin. HEK-293 cells stably expressing recombinant human A_{2B} AdoRs (HEK-h A_{2B} AdoR) were grown in DMEM supplemented with 10% fetal bovine serum and 0.5 mg/mL G-418. CHOK1 cells stably expressing the recombinant human A_1 AdoR (CHO-h A_1 AdoR) and A_3 AdoR (CHO-h A_3 AdoR) were grown as monolayers on 150-mm plastic

culture dishes in Ham's F-12 media supplemented with 10% fetal bovine serum in the presence of 0.5 mg/mL G-418. Cells were cultured in an atmosphere of 5% CO₂/95% air maintained at 37°C.

To make membranes, cells were detached from the culture plates into ice-cold 50 mmol/L Tris-HCl buffer (pH7.4). The cell suspensions were homogenized with Polytron at setting 4 for 30 seconds, and spun at 48,000g for 15 minutes. The pellets were washed three times by re-suspension in ice-cold Tris-HCl buffer and centrifugation. The final pellet was re-suspended in a small volume of Tris-HCl, aliquoted and frozen at -80°C until used for receptor binding assays. The protein concentration of membrane suspensions was determined using the Bradford method (Bio-Rad) with bovine serum as standards.

Competition Binding Assays- Competition assays were performed to determine the affinities (K_i) of the following unlabeled compounds (competing agents): Compounds WRC-0470; Compound 16 of this invention, NECA, CGS 21680 and R-PIA for A₁AdoRs ([³H]DPCPX binding sites on CHO-hA₁AdoR cell membranes), A_{2A}AdoRs([³H]ZM241385 binding sites on PC12 and HEK-hA_{2A}AR cell membranes),

A_{2B}AdoR ([³H]DPCPX binding sites on HEK-hA_{2B}AdoR cell membranes) and A₃AdoR ([¹²⁵I]ABMECA binding sites on CHO-hA₃AdoR cell membrane).

Membrane suspensions were incubated for 2 hours at room temperature in 50 mmol/L Tris-HCl buffer (pH 7.4) containing ADA (1 U/mL), Gpp(NH)p (100 μM), radioligand {either [³H]ZM241385 (-1.5 to 5 nmol/L), [³H]DPCPX (~2.5 to 3.0 nmol/L for A₁ and 30 nM for A_{2B}) or [¹²⁵I]ABMECA (1 nM)} and progressively higher concentrations of the competing agents. At the end of incubation, bound and free radioligands were separated by filtration through Whatman GF/C glass fiber filters using a Brandel tissue harvester (Gaithersburg, MD). Triplicate determinations were performed for each concentration of the competing agent.

Study Design (Protocols)

The affinity (K_i) of various CVT compounds for the A₁ and A_{2A} adenosine receptor were determined by their potency to compete for [³H]CPX (A₁) or [³H]ZM241385 (A_{2A}) binding sites on membranes derived from CHO-hA₁AdoR, PC12 or HEK-HA_{2A}AdoR cells. R-PIA and CGS21680, agonists that are selective for A₁ and A_{2A} respectively, and NECA, a non-selective AdoR agonist were used as controls. To facilitate comparison and avoid the complication of multiple affinity states due to receptor coupling to G-proteins, the competition binding studies were carried out in the presence of Gpp (NH) p (100 μM) to

uncouple receptors from G-proteins. The affinity of selected compounds for A_{2B} and A_3 receptors were assessed by their potencies to compete for [3H] CPX (A_{2B}) and [^{125}I] ABMECA (A_3) binding sites on membranes derived from HEK-h A_{2B} AdoR and CHO-h A_3 AdoR cells, respectively.

5 Results

The affinity (K_i) of WRC-0470; and Compound 16 for human A_1 , rat and human A_{2A} AdoRs, as determined by competition binding studies are summarized in Table 2, below. All compounds show moderate selectivity for human A_{2A} versus A_1 receptor. Furthermore, Compound 16, at a concentration of 10 μM , decreased the specific binding of [3H] CPX (HEK-h A_{2B} AdoR) or [^{125}I] IBMECA (CHO-h A_3 AdoR) by 20% and 22%, respectively.

Table 2. Binding Affinities of Adenosine Receptor Agonists for A_{2A} AdoRs and A_1 AdoRs
K_i/ nmol/L (pK_i±SEM)

	HEK-h A_{2A} AR Cells		CHO-h A_1 AR	
	Binding Affinity	n	Binding Affinity	n
WRC-0470	272 (6.55±0.04) [0.83±0.07]	6	7278 (5.16±0.09) [1.13±0.21]	3
Compound 16	1269 (5.90±0.03) [0.73±0.04]	7	>16460 (4.59±0.35) [0.92±0.04]	3
CGS21680	609 (6.22±0.06) [0.65±0.07]	3	>3540 (5.47±0.20)	3
NECA	360 (6.45±0.06) [0.83±0.08]	3	328 (6.49±0.06) [0.88±0.03]	3
R-PIA	1656 (5.78±0.02) [1.05±0.02]	3	477 (6.35±0.11) [1.03±0.08]	3

15 The results of this Experiment show that Compound 16 is a low affinity A_{2A} agonist.

Example 16

The objective of this Example was to characterize pharmacologically the effects of Compound 16 of this invention on coronary artery conductance. Specifically, the experiments were designed to determine 1) the potency Compound 16 and compared its potency to that of adenosine and other selected A_{2A} AdoR agonists, and 2) which adenosine receptor, the A_1 or A_{2A} AdoR subtype mediates the coronary vasodilation caused by Compound 16 of this invention.

In the heart, the A_{2A} adenosine receptor mediates the coronary vasodilation caused by adenosine, whereas the A_1 receptor mediates the cardiac depressant actions of adenosine, such as the negative chronotropic and dromotropic (AV block) effects.

Several potent and selective ligands, both agonists and antagonists, for the A_1 and A_{2A} AdoRs have been synthesized. In the heart agonists of A_1 AdoRs have been proposed to be useful as antiarrhythmic agents, whereas agonists of A_{2A} AdoRs are being developed for selective coronary vasodilation

A series of adenosine derivatives targeted for selective activation of A_{2A} adenosine receptor (A_{2A} AdoR) were synthesized for the purposes of developing coronary vasodilators. More specifically, in this study we report on the effect of a series of novel A_{2A} AdoR agonists on coronary artery conductance (vasodilation) in rat and guinea pig isolated perfused hearts.

Materials

Rats (Sprague Dawley) and Guinea pigs (Hartley) were purchased from Simonsen and Charles Rivers, respectively. WRC-0470 was prepared as described in the literature (K. Niiya et al., J. Med. Chem. 35; 4557-4561 (1992)). Compound 16 of this invention was prepared as described above. CGS 21680 and adenosine were purchased from Sigma. Krebs-Henseleit solution was prepared according to Standard Methods, and 0.9% saline was purchased from McGraw, Inc..

Methods

Adult Sprague Dawley rats and Hartley guinea pigs of either sex weighing from 230 to 260 grams and 300 to 350 grams, respectively were used in this study. Animals were anesthetized by peritoneal injection of a cocktail containing ketamine and xylazine (ketamine 100 mg, xylazine 20 mg/ml). The chest was opened and the heart quickly removed. The heart was briefly rinse in ice-cold Krebs-Henseleit solution (see below), and the aorta cannulated. The heart was then perfused at a flow rate of 10 ml/min with modified Krebs-Henseleit (K-H) solution containing NaCl 117.9, KCl 4.5, $CaCl_2$ 2.5, $MgSO_4$ 1.18, KH_2PO_4 1-

18, pyruvate 2.0 mmol/L. The K-H solution (pH 7.4) was gassed continuously with 95% O₂ and 5% CO₂ and warmed to 35±0.50 C. The heart was electrically paced at a fixed cycle length of 340 ms (250 beats/min) using a bipolar electrode placed on the left atrium. The electrical stimuli were generated by a Grass stimulator (Model S48, W. Warwick, RI) and delivered through a Stimuli Isolation Unit (Model SIU5, Astro-Med, Inc., NY) as square-wave pulses of 3-msec in duration and amplitude of at least twice the threshold intensity.

Coronary perfusion pressure (CPP) was measured using a pressure transducer, connected to the aortic cannula via a T-connector positioned approximately 3 cm above the heart. Coronary perfusion pressure was monitored throughout the experiment and recorded either on a chart recorder (Gould Recorder 2200S) or a computerized recording system (PowerLab/4S, ADInstruments Pty Ltd, Australia). Only hearts with CPP ranging from 60 to 85 mmHg (in the absence of drugs) were used in the study. Coronary conductance (in ml/min/mmHg) was calculated as the ratio between coronary perfusion rate (10 ml/min) and coronary perfusion pressure.

In experiments in which A₁ adenosine receptor-mediated negative dromotropic effect was measured, atrial and ventricular surface electrograms were recorded during constant atrial pacing. The effect of various adenosine receptor agonists on atrioventricular conduction time was determined as described previously by Jenkins and Belardinelli *Circ. Res.* 63: 97-116 (1988).

Stock solutions of Compound 16 of this invention (5mM) and CGS 21680 (5mM) were prepared in dimethyl sulfoxide (DMSO); purchased from Aldrich, PS 04253MS. A stock solution of adenosine (1 mg/ml) was prepared in saline. One concentration was made from the stock solution by dilution into saline to yield solution of either 2X10⁻⁴ or 2X10⁻⁵ M. These solutions were injected into the perfusion line of the apparatus as boluses of 20 µl. In some experiments the solutions were placed into a 30 ml glass syringe and the drugs were infused at rates necessary to achieve the desired perfusate concentrations (e.g, 10, 100 nM, etc).

Coronary vasodilation of A_{2A} adenosine receptor agonists

Concentration-response relationships for the effect of Compound 16 of this invention (0.1 to 400 nM) and CGS21680 (0.1 to 10 nM) to increase coronary conductance were obtained. After control measurements of coronary perfusion pressure were recorded, progressive higher concentrations of the adenosine receptor agonists were administered until maximal coronary vasodilation was observed. The steady-state responses to each concentration of adenosine

receptor agonists were recorded. In each heart of this series (4 to 6 hearts for each agonist) only one agonist and one concentration-response relationship was obtained.

Coronary vasodilatory effect of Compound 16 in the absence and presence of adenosine receptor antagonists.

5 To determine which adenosine receptor subtype (A_1 or A_{2A}) mediates the coronary vasodilation caused by Compound 12, the A_1 and A_{2A} adenosine receptor antagonists CPX and ZM241385, respectively, were used. Hearts ($n=6$) were exposed to the compound being tested (10 nM), and after the effect of this agonist reached steady-state, first CPX (60 nM), and then ZM241385 were added to the perfusate and the changes in CPP were recorded.

10 In isolated perfused hearts ($n=36$ rats and 18 guinea pigs) paced at constant atrial cycle length of 340 msec, adenosine, CGS21680, WRC0470, and Compound 16 caused a concentration-dependent increase in coronary conductance. CGS21680 and WRC0470 were the most potent agonists tested. Compound 16 was approximately 10-fold more potent than adenosine to increase coronary conductance. It is worth noting that all agonists were several
15 fold more potent coronary vasodilators in rat than guinea pig hearts (Table 3).

Table 3 - Potency of Adenosine and A_{2A} Adenosine Receptor Agonists to Increase Coronary Conductance in Rat and Guinea Pig Isolated Perfused Hearts

Potency (EC_{50})			
Agonist	n	Rat	Guinea Pig
Compound 16	4	6.4 ± 1.2	18.6 ± 6.0
Adenosine	4	59.2 ± 6.4	86.0 ± 0.5
CGS21680	4	0.5 ± 0.1	1.7 ± 0.4
WRC0470	3	0.6 ± 0.2	2.4 ± 1.1

20 To determine the AdoR subtype (A_1 versus A_{2A}) that is responsible for the coronary vasodilation observed in the presence of Compound 16, the effect of this agonist (10 nM) on coronary conductance was studied in the absence and presence of CPX, a selective A_1 AdoR antagonist (Belardinelli et al, 1998) and ZM241385, a selective A_{2A} AdoR antagonist (Poucher et al, 1995) at the concentration of 60nM. As shown in Figure 1, Compound 16 significantly increased coronary conductance to 0.22 ± 0.01 ml/mm Hg⁻¹ min⁻¹ from a baseline
25 value of 0.16 ± 0.02 mlmmHg⁻¹ min⁻¹. This increase in coronary conductance caused by Compound 16 was not affected by CPX but was completely reversed by ZM241385 (0.17 ± 0.02 mlmm Hg⁻¹ min⁻¹).

Example 17

The objective of this Example was to determine the functional selectivity of Compound 16 to cause coronary vasodilation. Specifically, the potency of Compound 16 to cause coronary vasodilation (A_{2A} AdoR response) and prolongation of A-V nodal conduction time (A_1 AdoR response) were determined in rat and guinea pig hearts.

Materials

Sprague Dawley rats were purchased from Simonsen. Hartley guinea pigs were purchased from Charles River. Compound 16 was prepared as described above. CVT-510 - 2-{6-[[[(3R)oxolan-3-yl]amino]purin-9-yl]}(4S,3R,5R)-5-(hydroxymethyl)oxolane-3,4-diol - was prepared in accordance with the synthesis method disclosed in U.S. Patent No. 5,789,416, the specification of which is incorporated herein by reference. Ketamine was purchased from Fort Dodge Animal Health (Lot No. 440444) and xylazine from Bayer (Lot No. 26051 A). Krebs-Henseleit solution was prepared according to the standard methods, and 0.9% sodium chloride was purchased from McGraw, Inc. (Lot No. J8B246).

Isolated perfused heart preparation:

Rats and guinea pigs, of either sex weighing from 230 to 260 grams and 300 to 350 grams, respectively, were used in this study. Animals were anesthetized by peritoneal injection of a cocktail containing ketamine and xylazine (ketamine 100 mg, xylazine 20 mg/ml). The chest was opened and the heart quickly removed. The heart was briefly rinse in ice-cold Krebs-Henseleit solution (see below), and the aorta cannulated. The heart was then perfused at a flow rate of 10 ml/min with modified Krebs-Henseleit (K-H) solution containing NaCl 117.9, KCl 4.5, CaCl_2 2.5, MgSO_4 1.18, KH_2PO_4 1.18, pyruvate 2.0 mmol/L. The K-H solution (pH 7.4) was gassed continuously with 95% O_2 and 5% CO_2 and warmed to $35 \pm 0.50^\circ\text{C}$. The heart was electrically paced at a fixed cycle length of 340 ms (250 beats/min) using a bipolar electrode placed on the left atrium. The electrical stimuli were generated by a Grass stimulator (Model S48, W. Warwick, RI) and delivered through a Stimuli Isolation Unit (Model SIU5, Astro-Med, Inc., NY) as square-wave pulses of 3-msec in duration and amplitude of at least twice the threshold intensity.

Coronary perfusion pressure (CPP) was measured using a pressure transducer, connected to the aortic cannula via a T-connector positioned approximately 3 cm above the heart. Coronary perfusion pressure was monitored throughout the experiment and recorded either on a chart recorder (Gould Recorder 2200S) or a computerized recording system (PowerLab/4S, ADInstruments Pty Ltd, Australia). Only hearts with CPP ranging from 60 to

85 mmHg (in the absence of drugs) were used in the study. Coronary conductance (in ml/min/mmHg) was calculated as the ratio between coronary perfusion rate (10 ml/min) and coronary perfusion pressure.

A_1 adenosine receptor-mediated depression of A-V nodal conduction time (negative dromotropic effect) was measured. Atrial and ventricular surface electrograms in rats and His bundle electrogram in guinea pigs, were recorded during constant atrial pacing. The effects of Compound 16 on atrioventricular conduction time and stimulus-to-His-bundle (S-H interval) were determined as described previously by Jenkins and Belardinelli (1988).

The effects of Compound 16 on coronary conductance (A_{2A} effect) and atrioventricular conduction time or stimulus-to-His-bundle (S-H) interval (A_1 effect) was then determined. Hearts were instrumented for continuous recording of coronary perfusion pressure (A_{2A} response) and atrioventricular (A-V) conduction time or S-H interval (A_1 response). In each experiment, concentration-response relationship of Compound 16 (n=6 rats, 4 guinea pigs) to increase coronary conductance and to prolong A-V conduction time or S-H interval was determined. After control measurements of CPP and A-V conduction time or S-H interval were made, progressive higher concentrations of Compound 16 was administered until maximal coronary vasodilation and A-V nodal conduction time or S-H interval prolongation were achieved. In separate rat hearts (n=4), the effect of various concentrations (100 - 400 nM) of CVT510, an A_1 adenosine agonist (Snowdy et al, 1999), on A-V nodal conduction time was determined and compared to that of Compound 16 (0.1- 30 μ M).

The concentration-response curves for Compound 16 to increase coronary artery conductance and to prolong A-V nodal conduction time or S-H interval are shown in Figures 2 and 3. In both rat and guinea pig, Compound 16 increased coronary conductance in a concentration dependent manner. The potencies (EC_{50} values) for Compound 16 to increase coronary conductance in rat hearts was 6.4 ± 0.6 nM and in guinea pig hearts was 18.6 ± 6.0 nM. In contrast, the effect of this agonist on S-H interval was somewhat variable between rat and guinea pig hearts. In rat hearts Compound 16 did not prolong A-V nodal conduction time (Figure 2 and 3) whereas the A_1 AdoR agonist CVT510 significantly prolonged the A-V nodal conduction time (Figure 4). Unlike in rat, in guinea pig hearts Compound 16 caused a concentration-dependent prolongation of S-H interval (A_1 response) with an EC_{50} value (potency) of 4.0 ± 2.3 μ M (Figure 4). This latter value is approximately 215-fold greater (i.e., less potent) than the EC_{50} value of 18.6 ± 6.0 nM to cause coronary vasodilation (A_{2A} response- Figure 3).

The results indicate that Compound 16 is a coronary vasodilator (A_{2A} AdoR-mediated effect) devoid of negative dromotropic effect (A_1 AdoR-mediated effect) in rat hearts. In guinea pig hearts Compound 16 caused some negative dromotropic effect. Nevertheless, Compound 16 was at least 215-fold more selective to cause coronary vasodilation than negative dromotropic effect. The reason(s) for the species difference in the A_1 AdoR-mediated response elicited by Compound 16 is unknown. Regardless, in both species (rat and guinea pig) Compound 16 causes maximal coronary vasodilation at concentrations that do not cause prolongation of A-V nodal conduction time, i.e., without negative dromotropic effect. It was also observed that Compound 16 has a greater affinity (i.e., >2- / >-13-fold) for A_{2A} than A_1 AdoR and that there is a markedly greater receptor reserve for A_{2A} AdoR-mediated coronary vasodilation than for A_1 AdoR-mediated negative dromotropic effect.

Example 18

The present study was designed to test the hypothesis that there is an inverse relationship between the affinity (K_i or pK_i) and duration of action of A_{2A} adenosine receptors (AdoR). Specifically, the aims of the study were to determine the relationship between the duration of the coronary vasodilation caused by a selected series of high and low affinity A_{2A} AdoR agonists in rat isolated hearts and anesthetized pigs; and the affinity of these agonists for A_{2A} AdoRs in pig striatum.

Materials: Rats (Sprague Dawley) were purchased from Simonen. Farm pigs were obtained from Division of Laboratory Animal Resources, University of Kentucky. Compound 12, Compound 13, and Compound 16 of this invention were prepared as described in the methods above. YT-0146 was prepared as described in U.S. Patent No. 4,956,345, the specification of which is incorporated herein by reference. WRC-0470 was prepared as described in the literature (K. Niiya et al., J. Med. Chem. 35; 4557-4561 (1992). CGS21680 was purchased from Research Biochemicals, Inc. and Sigma and R-PIA (Lot No. WY-V-23) was purchased from Research Biochemicals, Inc. HENECA was a gift from Professor Gloria Cristalli of University of Camerino, Italy.

The anesthetic agents: Ketamine was purchased from Fort Dodge Animal Health. Xylazine was purchased from Bayer. Sodium pentobarbital was purchased from The Butler Co. Phenylephrine was purchased from Sigma. DMSO was purchased from Sigma and American Tissue Type Collections. Krebs-Henseleit solution was prepared according to standard methods, and 0.9% saline was purchased from McGraw, Inc.

In this study, the following laboratory preparations were used. 1) Rat isolated perfused hearts; 2) Anesthetized open-chest pigs;

Rat isolated perfused heart preparation

Adult Sprague Dawley rats of either sex weighing from 230 to 260 grams were used in this study. Animals were anesthetized by peritoneal injection of a cocktail containing ketamine and xylazine (ketamine 100 mg, xylazine 20 mg/ml). The chest was opened and the heart quickly removed. The heart was briefly rinse in ice-cold Krebs-Henseleit solution (see below), and the aorta cannulated. The heart was then perfused at a flow rate of 10 ml/min with modified Krebs-Henseleit (K-H) solution containing NaCl 117.9, KCl 4.5, CaCl. 2.5, $MgSO_4$ 1.18, KH_2PO_4 1.18, pyruvate 2.0 mmol/L. The K-H solution (pH 7.4) was gassed continuously with 95% O_2 and 5% CO_2 and warmed to $35 \pm 0.50^\circ C$. The heart was electrically paced at a fixed cycle length of 340 ms (250 beats/min) using a bipolar electrode place on the

left atrium. The electrical stimuli were generated by a Grass stimulator (Model S48, W. Warwick, RI) and delivered through a Stimuli Isolation Unit (Model SIU5, Astro-Med, Inc., NY) as square-wave pulses of 3msec in duration and amplitude of at least twice the threshold intensity.

5 Coronary perfusion pressure (CPP) was measured using a pressure transducer, connected to the aortic cannula via a T-connector positioned approximately 3 cm above the heart. Coronary perfusion pressure was monitored throughout the experiment and recorded either on a chart recorder (Gould Recorder 2200S) or a computerized recording system (PowerLab/4S, ADInstruments Pty Ltd, Australia). Only hearts with CPP ranging from 60 to
10 85 mmHg (in the absence of drugs) were used in the study. Coronary conductance (in ml/min/mmHg) was calculated as the ratio between coronary perfusion rate (10 ml/min) and coronary perfusion pressure.

Anesthetized open-chest pig preparation

15 Farm pigs weighing 22-27 kg were used in this study. All animals received humane care according to the guidelines set forth in "The Principles of Laboratory Animal Care" formulated by the National Society for Medical research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996). In addition, animals were used in accordance with the guidelines of the University of Kentucky
20 Institutional Animal Care and Use Protocol.

 Anesthesia was anesthetized with ketamine (20 mg/kg, i.m.) and sodium pentobarbital (15-18 mg/kg i.v.). Anesthesia was maintained with additional sodium pentobarbital (1.5-2 mg/kg, i.v.) every 15-20 minutes. Ventilation was maintained via a tracheotomy using a mixture of room air and 100 % O₂. Tidal volume, respiratory rate and fraction of O₂ in
25 inspired air were adjusted to maintain normal arterial blood gas (ABG) and pH values. Core body temperature was monitored with an esophageal temperature probe and maintained with a heating pad between 37.0-37.5°C. Lactate Ringers solution was administered via an ear or femoral vein, at 5-7 ml/kg/min after a initial bolus of 300-400 ml. A catheter was inserted into the femoral artery to monitor arterial blood pressure and to obtain ABG samples.

30 The heart was exposed through a median sternotomy, and suspended in a pericardial cradle. Left ventricular pressure (LVP) was measured with a 5F high fidelity pressure sensitive tip transducer (Millar Instruments, Houston, TX) placed in the left ventricular cavity via the apex and secured with a purse string suture. A segment of the left anterior descending coronary artery (LAD), proximal to the origin of the first diagonal branch, was dissected free

of, surrounding tissue. A transit time perivascular flow probe (Transonic Systems Inc., Ithaca, NY) was placed around this segment to measure coronary blood flow (CBF). Proximal to the flow probe a 24g modified angiocatheter was inserted for intracoronary infusions. All hemodynamic data were continuously displayed on a computer monitor and fed
5 through a 32 bit analog-digital converter into an online data acquisition computer with customized software (Augury, Coyote Bay Instruments, Manchester, NH). A_{2A} AdoR agonists were dissolved in DMSO to produce stock concentrations of 1-5 mM, which were diluted in 0.9% saline and infused at rates of 1-1.5 ml/min. The A_{2A} AdoR agonists were administered intracoronary. To maintain blood pressure constant, phenylephrine was
10 administered intravenously. The phenylephrine stock solution (30 mM) was prepared in distilled water.

Isolated perfused hearts

To determine the duration of the A_{2A} adenosine receptor mediated coronary vasodilation caused by adenosine and adenosine receptor agonists, the agonists were administered
15 intervencously either by bolus injection (protocol A) or by continuous infusion (protocol B). Protocol A: Bolus injections. In each heart of this series (3 to 11 hearts for each agonist), boluses of adenosine (20 μ l, 2×10^{-4} M), Compounds of this invention (20 to 40 μ l, 2×10^{-5} M), and other adenosine receptor agonists were injected into the perfusion line. The times to 50% ($t_{0.5}$) and 90% ($t_{0.9}$) reversal of the decrease in CPP were measured. Each heart was
20 exposed to a maximum of three vasodilators.

Protocol B: Continuous infusion. In a separate series of experiments (n=4), Compound 16 and adenosine were infused into the perfusion line at constant rate for a period of six minutes. The perfusate concentrations of Compound 16 and adenosine were 20 nM and 200 nM respectively, which were approximately 4x their respective concentrations previously
25 established to cause 50% of maximal increase in coronary conductance (EC_{50}) in rat isolated perfused hearts. The times to 50% ($t_{0.5}$) and 90% ($t_{0.9}$) reversal of the decreases in CPP were measured from the time at which the infusion of the agonists was stopped.

Dose-dependent duration of maximal vasodilation caused by bolus injections of Compound 16. To determine the dependency of the duration of maximal coronary
30 vasodilation on the dose of Compound 16, boluses (100 – 300 μ l) of a 2×10^{-5} M stock solution of Compound 16 were injected into the perfusion line. In addition, the duration of the injection was varied according to the volume of the boluses such as 10, 20 and 30 sec for 100, 200 and 300 μ l boluses respectively. The duration of maximal effect was measured

from the point at which the decrease in CPP reached the nadir to the onset point of reversal of CPP.

Relationship between affinity of various agonists for A_{2A} adenosine receptor and the reversal time of their effect to increase coronary conductance: These experiments were performed to construct the relationship between the affinities of the various agonists for A_{2A} adenosine receptor and the duration of their respective effect on coronary conductance. Boluses of various agonists were injected into the perfusion line of rat isolated perfused hearts (n=4 to 6 for each agonist) and the time to 90% (*t* 0.9) reversal of the decrease in CPP measured. The affinities of the various agonists for A_{2A} adenosine receptor was determined in pig striatum membranes using a radioligand binding assay, as described above. The reversal time (*t* 0.9) of the decrease in CPP was plotted against their affinities (*pK_i*) for the A_{2A} adenosine receptor.

Open-chest pig

Prior to initiating the experiment, a 30-minute stabilization period followed the completion of all instrumentation. After obtaining the baseline hemodynamic data the first intracoronary infusion of an A_{2A} ADOR agonist was initiated. Infusions were maintained for 4-5 minutes to allow LAD CBF to reach a steadystate, after which the infusion was terminated. The time to recovery of 50% (*t* 0.5) and 90% (*t* 0.9) of baseline CBF were recorded. Ten to 15 minutes after CBF returned to pre-drug values a second infusion with a different agonist was started. In preliminary studies it was found that the intracoronary infusion of adenosine agonists produced varying degrees of systemic hypotension, and hence, in all subsequent experiments, phenylephrine was administered intravenously. Hemodynamic measurements were made prior to and following the initiation of the phenylephrine infusion at dose of - 1 µg/kg/min. The phenylephrine infusion rate was adjusted during and following the infusions of the adenosine agonists to maintain arterial blood pressure within 5 mmHg of preinfusion values. The effect of a maximum of three different agonists was determined in each experiment.

Results

Adenosine, the compounds of this invention and other adenosine derivatives were given as boluses into the perfusion line at concentrations that cause equal or near-equal increases in coronary conductance. Although adenosine and the agonists caused equal maximal increases in coronary conductance the duration of their effect was markedly different. The duration of the effect of adenosine was the shortest followed by Compound 16,

whereas that of CGS21680 and WRC0470 were the longest. The durations of the coronary vasodilation caused by adenosine, the compounds of this invention and other agonists measured as the time to 50% and 90% ($t_{0.5}$ and $t_{0.9}$, respectively) reversal of the increases in coronary conductance are summarized in Table 4

5 **Table 4 – Reversal Time Of Coronary Vasodilation by Adenosine and adenosine receptor agonists in Rat Isolated Perfused Hearts**

Agonist	$t_{0.5}$ (min)	$t_{0.9}$ (min)	n
Adenosine	1.06±0.1	5.6±0.8	11
HENECA	28.6±1.1	32.8±3.1	3
R-PIA	7.9±0.1	12.6±0.8	3
CGS21680	14.5±0.9	19.5±0.9	3
YT-146	17.7±1.0	28.5±4.0	3
Compound 12	14.83±2.1	15.0±0.8	3
Compound 13	14.4±1.9	21.3±3.9	4
Compound 16	5.2±0.2	11.3±1.1	5

Time (in minutes) to 50% and 90% ($t_{0.5}$ and $t_{0.9}$, respectively) reversal of the increases in coronary conductance caused by adenosine and adenosine receptor agonists. Values are the means±SEM of single determinations in each of the preparations (n).

10

The reversal time of coronary vasodilation was dependent on the affinity of the adenosine derivatives for brain striatum A_{2A} receptors. (Fig. 2A) There was a significant ($P < 0.05$) inverse relationship ($r = 0.87$) between the affinity (PK_i) of the agonists for the A_{2A} AdoR and the reversal time ($t_{0.9}$) of the coronary vasodilation caused by the same agonists.

15

Regardless of whether Compound 16 was given as bolus or continuous infusion the reversal of the coronary vasodilation was relatively rapid. In fact, a comparison between a six minute infusion of adenosine and Compound 16 at doses that they cause equal decreases in coronary perfusion pressure (CPP) revealed that adenosine and Compound 16 have a similar time course for vasodilation and reversal time. Both the $t_{0.5}$ and $t_{0.9}$ were near identical.

20

The duration of the coronary vasodilation by Compound 16 was dose-dependent. Increasing the volume of a bolus of Compound 16 (stock solution of 2×10^{-5} M) caused progressively longer lasting decreases in CPP. The maximal duration of the coronary vasodilation (time that CPP remained at its lowest) increased as the volume of the boluses increased from 100 μ l to 200 and 300 μ l without affecting the maximal decreases in CPP.

Coronary vasodilation in an open-chest pig preparation

In *in situ* hearts of an open-chest anesthetized pig preparation Compound 16 of this invention as well as CGS21680 and other A_{2A}AdoR agonists (i.e., WRC-0470 and YT-146) caused significant increases in coronary blood flow (CBF). Selected doses of these compounds given as continuous (4 to 5 min) intracoronary infusions caused 3.1 to 3.8-fold increases in CBF as set forth in Table 3, below. Once established that all agonists caused near the same magnitude of increases in CBF (i.e., "fold increase") and cause similar changes in heart rate and mean arterial blood pressure, the reversal time of their respective coronary vasodilation effects was determined.

10 **Table 5. Magnitude of Increase in Coronary Blood Flow Caused by Various Adenosine Receptor Agonists in Open-Chest Anesthetized Pigs**

Agonist	CBF ("Fold Increase")	n
Compound 16 (10µg/kg/min)	3.40±0.04	3
Compound 16 (310µg/kg/min)	3.83±0.39	6
WRC-470 (1µg/kg/min)	3.14±0.24	6
GSC21680 (2µg/kg/min)	3.54±0.093	3
YT-146 (1µg/kg/min)	3.44±0.47	3

Maximal "fold-increase" in coronary blood flow (CBF) above baseline caused by various adenosine receptor agonists. Data represent mean ± SEM of one or two measurements in each pig (n).

15 As summarized in Table 6 the t_{0.5} and t_{0.9} of coronary vasodilation caused by the various A_{2A} AdoR agonists and "CVT-compounds" was variable. The reversal time of the increase in CBF caused by Compound 16 of this invention were shorter than that of CGS21680, WRC-0470 or YT-146. More importantly, as in rat isolated perfused hearts, there was a significant (P < 0.05) inverse relationship (r = 0.93) between the affinity (PKi) of the A_{2A}AdoR agonists for pig brain striatum A_{2A} receptors and the reversal time (t_{0.9}) of coronary vasodilation. There was an excellent concordance between the reversal time of the coronary vasodilation caused by a selected number of agonists in rat isolated perfused hearts and in anesthetized open chest pig preparations.

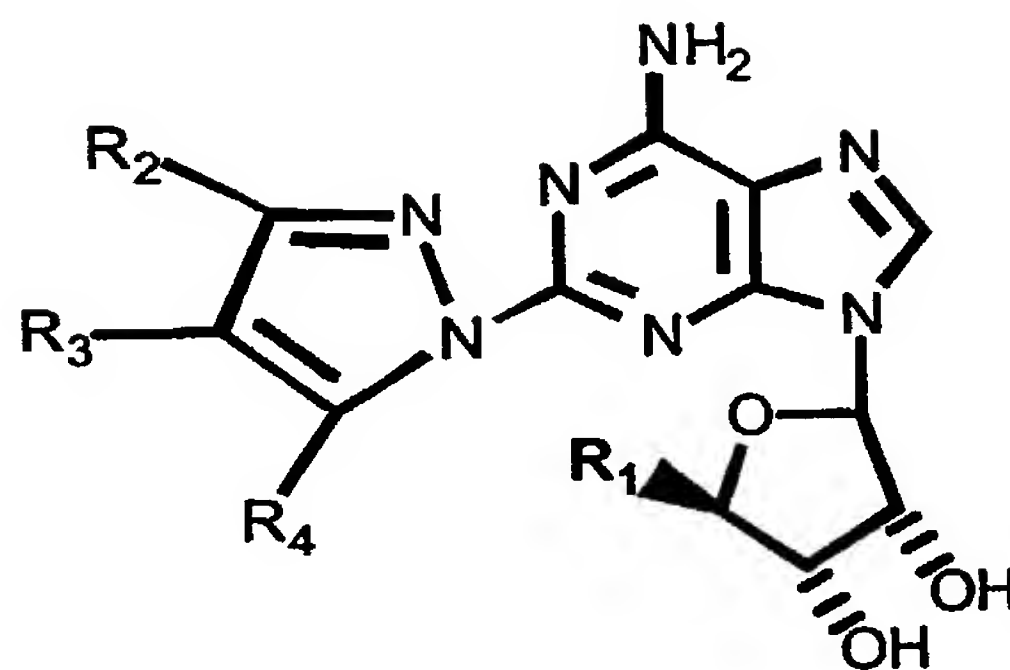
25 **Table 6 - Reversal Time of Coronary Vasodilation Caused by Various Adenosine Receptor Agonists in Open-Chest Anesthetized Pigs**

Agonist	t _{0.5} (min)	t _{0.9} (min)	n
Compound 16 (10µg/kg/min)	1.9±0.2	10.1±0.7	3
Compound 16 (310µg/kg/min)	2.6±0.4	12.3±1.1	6

Compound 16 is a low affinity A_{2A} AdoR agonists and less potent (- 10-fold) than the prototypical agonist CGS21680. Nevertheless Compound 16 is a full agonist to cause coronary vasodilation. But, as shown in this study the duration of its effect is several-fold shorter than that of the high affinity agonists CGS21680 and WRC-0470. Hence, Compound 16 is a short acting A_{2A} AdoR agonists coronary vasodilator. Because of its short duration of action in comparison to the high affinity A_{2A} AdoR agonists (e.g., WRC-0470, CGS21680) this low affinity but still full agonist coronary vasodilator may prove to be ideal pharmacological "stressor agents" during radionuclide imaging of the myocardium.

What we claim is:

- 1. A compound having the formula:**



wherein $R^1 = CH_2OH, -CONR_5R_6$;

5 R^3 is selected from the group consisting of C_{1-15} alkyl, halo, NO_2 , CF_3 , CN , OR^{20} , SR^{20} , $N(R^{20})_2$, $S(O)R^{22}$, SO_2R^{22} , $SO_2N(R^{20})_2$, $SO_2NR^{20}COR^{22}$, $SO_2NR^{20}CO_2R^{22}$, $SO_2NR^{20}CON(R^{20})_2$, $N(R^{20})_2NR^{20}COR^{22}$, $NR^{20}CO_2R^{22}$, $NR^{20}CON(R^{20})_2$, $NR^{20}C(NR^{20})NHR^{23}$, COR^{20} , CO_2R^{20} , $CON(R^{20})_2$, $CONR^{20}SO_2R^{22}$, $NR^{20}SO_2R^{22}$, $SO_2NR^{20}CO_2R^{22}$, $CONR^{20}SO_2R^{22}$, $OC(O)R^{20}$, $C(O)OCH_2OC(O)R^{20}$, and $OCON(R^{20})_2$, $-CONR^7R^8$, C_{2-15} alkenyl, C_{2-15} alkynyl, heterocyclyl, aryl, and heteroaryl, wherein the alkyl, alkenyl, alkynyl, aryl, heterocyclyl and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, alkyl, NO_2 , heterocyclyl, aryl, heteroaryl, CF_3 , CN , OR^{20} , SR^{20} , $N(R^{20})_2$, $S(O)R^{22}$, SO_2R^{22} , $SO_2N(R^{20})_2$, $SO_2NR^{20}COR^{22}$, $SO_2NR^{20}CO_2R^{22}$, $SO_2NR^{20}CON(R^{20})_2$, $N(R^{20})_2NR^{20}COR^{22}$, $NR^{20}CO_2R^{22}$, $NR^{20}CON(R^{20})_2$, $NR^{20}C(NR^{20})NHR^{23}$, COR^{20} , CO_2R^{20} , $CON(R^{20})_2$, $CONR^{20}SO_2R^{22}$, $NR^{20}SO_2R^{22}$, $SO_2NR^{20}CO_2R^{22}$, $CONR^{20}SO_2R^{22}$, $OC(O)R^{20}$, $C(O)OCH_2OC(O)R^{20}$, and $OCON(R^{20})_2$ and wherein optional heteroaryl, aryl, and heterocyclyl substituent is optionally substituted with halo, NO_2 , alkyl, CF_3 , amino, mono- or di- alkylamino, alkyl or aryl or heteroaryl amide, $NCOR^{22}$, $NR^{20}SO_2R^{22}$, COR^{20} , CO_2R^{20} , $CON(R^{20})_2$, $NR^{20}CON(R^{20})_2$, $OC(O)R^{20}$, $OC(O)N(R^{20})_2$, SR^{20} , $S(O)R^{22}$, SO_2R^{22} , $SO_2N(R^{20})_2$, CN , and OR^{20} ;

R⁵ and R⁶ are each individually selected from H, C₁-C₁₅ alkyl optionally substituted with from 1 to 2 substituents independently selected from the group consisting of halo, NO₂, heterocyclyl, aryl, heteroaryl, CF₃, CN, OR²⁰, SR²⁰, N(R²⁰)₂, S(O)R²², SO₂R²², SO₂N(R²⁰)₂, SO₂NR²⁰COR²², SO₂NR²⁰CO₂R²², SO₂NR²⁰CON(R²⁰)₂, N(R²⁰)₂NR²⁰COR²², NR²⁰CO₂R²², NR²⁰CON(R²⁰)₂, NR²⁰C(NR²⁰)NHR²³, COR²⁰, CO₂R²⁰, CON(R²⁰)₂, CONR²⁰SO₂R²², NR²⁰SO₂R²², SO₂NR²⁰CO₂R²², OCONR²⁰SO₂R²², OC(O)R²⁰, C(O)OCH₂OC(O)R²⁰, and

$\text{OCON(R}^{20})_2$ and wherein optional heteroaryl, aryl, and heterocyclyl substituent is optionally substituted with halo, NO_2 , alkyl, CF_3 , amino, mono- or di- alkylamino, alkyl or aryl or heteroaryl amide, NCOR^{22} , $\text{NR}^{20}\text{SO}_2\text{R}^{22}$, COR^{20} , CO_2R^{20} , $\text{CON(R}^{20})_2$, $\text{NR}^{20}\text{CON(R}^{20})_2$, OC(O)R^{20} , $\text{OC(O)N(R}^{20})_2$, SR^{20} , S(O)R^{22} , SO_2R^{22} , $\text{SO}_2\text{N(R}^{20})_2$, CN, and OR^{20} ;

5 R^7 is selected from the group consisting of hydrogen, C_{1-15} alkyl, C_{2-15} alkenyl, C_{2-15} alkynyl, heterocyclyl, aryl and heteroaryl, wherein the alkyl, alkenyl, alkynyl, aryl, heterocyclyl and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, NO_2 , heterocyclyl, aryl, heteroaryl, CF_3 , CN, OR^{20} , SR^{20} , $\text{N(R}^{20})_2$, S(O)R^{22} , SO_2R^{22} , $\text{SO}_2\text{N(R}^{20})_2$, $\text{SO}_2\text{NR}^{20}\text{COR}^{22}$,
 10 $\text{SO}_2\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CON(R}^{20})_2$, $\text{N(R}^{20})_2$ $\text{NR}^{20}\text{COR}^{22}$, $\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{NR}^{20}\text{CON(R}^{20})_2$, $\text{NR}^{20}\text{C(NR}^{20})\text{NHR}^{23}$, COR^{20} , CO_2R^{20} , $\text{CON(R}^{20})_2$, $\text{CONR}^{20}\text{SO}_2\text{R}^{22}$, $\text{NR}^{20}\text{SO}_2\text{R}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{OCONR}^{20}\text{SO}_2\text{R}^{22}$, OC(O)R^{20} , $\text{C(O)OCH}_2\text{OC(O)R}^{20}$ and $\text{OCON(R}^{20})_2$ and wherein optional heteroaryl, aryl and heterocyclyl substituent is optionally substituted with
 15 halo, NO_2 , alkyl, CF_3 , amino, mono- or di- alkylamino, alkyl or aryl or heteroaryl amide, NCOR^{22} , $\text{NR}^{20}\text{SO}_2\text{R}^{22}$, COR^{20} , CO_2R^{20} , $\text{CON(R}^{20})_2$, $\text{NR}^{20}\text{CON(R}^{20})_2$, OC(O)R^{20} , $\text{OC(O)N(R}^{20})_2$, SR^{20} , S(O)R^{22} , SO_2R^{22} , $\text{SO}_2\text{N(R}^{20})_2$, CN, and OR^{20} ;

R^8 is selected from the group consisting of hydrogen, C_{1-15} alkyl, C_{2-15} alkenyl, C_{2-15} alkynyl, heterocyclyl, aryl, and heteroaryl, wherein the alkyl, alkenyl, alkynyl, aryl, heterocyclyl, and heteroaryl substituents are optionally substituted with from 1 to 3
 20 substituents independently selected from the group consisting of halo, NO_2 , heterocyclyl, aryl, heteroaryl, CF_3 , CN, OR^{20} , SR^{20} , $\text{N(R}^{20})_2$, S(O)R^{22} , SO_2R^{22} , $\text{SO}_2\text{N(R}^{20})_2$, $\text{SO}_2\text{NR}^{20}\text{COR}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CON(R}^{20})_2$, $\text{N(R}^{20})_2$ $\text{NR}^{20}\text{COR}^{22}$, $\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{NR}^{20}\text{CON(R}^{20})_2$, $\text{NR}^{20}\text{C(NR}^{20})\text{NHR}^{23}$, COR^{20} , CO_2R^{20} , $\text{CON(R}^{20})_2$, $\text{CONR}^{20}\text{SO}_2\text{R}^{22}$, $\text{NR}^{20}\text{SO}_2\text{R}^{22}$, $\text{SO}_2\text{NR}^{20}\text{CO}_2\text{R}^{22}$, $\text{OCONR}^{20}\text{SO}_2\text{R}^{22}$, OC(O)R^{20} , $\text{C(O)OCH}_2\text{OC(O)R}^{20}$, and $\text{OCON(R}^{20})_2$ and
 25 wherein each optional heteroaryl, aryl, and heterocyclyl substituent is optionally substituted with halo, NO_2 , alkyl, CF_3 , amino, mono- or di- alkylamino, alkyl or aryl or heteroaryl amide, NCOR^{22} , $\text{NR}^{20}\text{SO}_2\text{R}^{22}$, COR^{20} , CO_2R^{20} , $\text{CON(R}^{20})_2$, $\text{NR}^{20}\text{CON(R}^{20})_2$, OC(O)R^{20} , $\text{OC(O)N(R}^{20})_2$, SR^{20} , S(O)R^{22} , SO_2R^{22} , $\text{SO}_2\text{N(R}^{20})_2$, CN, and OR^{20} ;

R^{20} is selected from the group consisting of H, C_{1-15} alkyl, C_{2-15} alkenyl, C_{2-15} alkynyl,
 30 heterocyclyl, aryl, and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heterocyclyl, aryl, and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from halo, alkyl, mono- or dialkylamino, alkyl or aryl or heteroaryl amide, CN, O- C_{1-6} alkyl, CF_3 , aryl, and heteroaryl;

R^{22} is selected from the group consisting of C_{1-15} alkyl, C_{2-15} alkenyl, C_{2-15} alkynyl,

heterocyclyl, aryl, and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heterocyclyl, aryl, and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from halo, alkyl, mono- or dialkylamino, alkyl or aryl or heteroaryl amide, CN, O-C₁₋₆ alkyl, CF₃, aryl, and heteroaryl; and

5 wherein R² and R⁴ are selected from the group consisting of H, C₁₋₆ alkyl and aryl optionally substituted with halo, CN, CF₃, OR²⁰ and N(R²⁰)₂, with the proviso that when R² is not hydrogen then R⁴ is hydrogen, and when R⁴ is not hydrogen then R² is hydrogen.

2. The compound of claim 1 wherein R³ is selected from the group consisting of C₁₋₁₅ alkyl, halo, CF₃, CN, OR²⁰, SR²⁰, S(O)R²², SO₂R²², SO₂N(R²⁰)₂, COR²⁰, CO₂R²⁰, -
10 CONR⁷R⁸, aryl and heteroaryl wherein the alkyl, aryl and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, aryl, heteroaryl, CF₃, CN, OR²⁰, SR²⁰, S(O)R²², SO₂R²², SO₂N(R²⁰)₂, COR²⁰, CO₂R²⁰ and CON(R²⁰)₂, and wherein each optional heteroaryl and aryl substituent is optionally substituted with halo, alkyl, CF₃, CN, and OR²⁰;

15 R⁵ and R⁶ are each individually selected from the group consisting of H, and C₁₋₁₅ alkyl optionally substituted with one aryl substituent that is optionally substituted with halo or CF₃;

R⁷ is selected from the group consisting of C₁₋₁₅ alkyl, C₂₋₁₅ alkynyl, aryl, and heteroaryl, wherein the alkyl, alkynyl, aryl, and heteroaryl substituents are optionally
20 substituted with from 1 to 3 substituents independently selected from the group consisting of halo, aryl, heteroaryl, CF₃, CN, and OR²⁰, and wherein each optional heteroaryl and aryl substituent is optionally substituted with halo, alkyl, CF₃, CN, and OR²⁰;

R⁸ is selected from the group consisting of hydrogen and C₁₋₁₅ alkyl;

R²⁰ is selected from the group consisting of H, C₁₋₄ alkyl and aryl, wherein the alkyl
25 and aryl substituents are optionally substituted with one alkyl substituent; and

R²² is selected from the group consisting of C₁₋₄ alkyl and aryl, wherein the alkyl and aryl substituents are optionally substituted with from 1 to 3 alkyl groups.

3. The compound of claim 1 wherein R³ is selected from the group consisting of C₁₋₁₅ alkyl, halo, CF₃, CN, OR²⁰, CO₂R²⁰, -CONR⁷R⁸, aryl and heteroaryl, wherein the alkyl,
30 aryl and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, alkyl, aryl, CF₃, CN, OR²⁰, CO₂R²⁰ or CON(R²⁰)₂, and wherein each optional heteroaryl and aryl substituent is optionally substituted with halo, alkyl, CF₃, CN, and OR²⁰;

R⁵ and R⁶ are each individually selected from hydrogen and C₁₋₆ alkyl;

R^7 is selected from the group consisting of C_{1-10} alkyl, aryl, and heteroaryl, wherein the alkyl, aryl and heteroaryl substituents are optionally substituted with from 1 to 2 substituents independently selected from the group consisting of halo, aryl, heteroaryl, CF_3 , CN, and OR^{20} , and wherein each optional heteroaryl and aryl substituent is optionally substituted with halo, alkyl, CF_3 , CN, and OR^{20} ;

R^8 is selected from the group consisting of hydrogen and C_{1-15} alkyl; and

R^{20} is selected from the group consisting of hydrogen and C_{1-4} alkyl.

4. The compound of claim 1 wherein R^3 is selected from the group consisting of C_{1-10} alkyl, halo, CF_3 , CN, CO_2R^{20} , $-CONR^7R^8$, aryl and heteroaryl wherein the alkyl, aryl and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, alkyl, CF_3 , CN, OR^{20} and $CON(R^{20})_2$;

R^5 and R^6 are each individually selected from hydrogen and C_{1-6} alkyl;

R^7 is selected from the group consisting of C_{1-10} alkyl, aryl, and heteroaryl, wherein the alkyl, aryl and heteroaryl substituents are optionally substituted with from 1 to 2 substituents independently selected from the group consisting of halo, aryl, heteroaryl, CF_3 , CN, OR^{20} and wherein each optional heteroaryl and aryl substituent is optionally substituted with halo, alkyl, CF_3 , CN, and OR^{20} ;

R^8 is selected from hydrogen and C_{1-15} alkyl; and

R^{20} is selected from hydrogen and C_{1-4} alkyl.

5. The compound of claim 1 wherein R^3 is selected from the group consisting of C_{1-10} alkyl, halo, CF_3 , CN, OR^{20} , CO_2R^{20} , $-CONR^7R^8$ and aryl; wherein the alkyl and aryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, alkyl, CF_3 , CN, OR^{20} and $CON(R^{20})_2$;

R^5 and R^6 are each individually selected from hydrogen and C_{1-6} ;

R^7 is selected from the group consisting of C_{1-10} alkyl, aryl, and heteroaryl, where the alkyl, aryl and heteroaryl substituents are optionally substituted with from 1 to 2 substituents independently selected from the group consisting of halo, aryl, heteroaryl, CF_3 , CN, OR^{20} and wherein each optional heteroaryl and aryl substituent is optionally substituted with halo, alkyl, CF_3 , CN, and OR^{20} ;

R^8 is selected from hydrogen and C_{1-15} alkyl; and

R^{20} is selected from hydrogen and C_{1-4} alkyl.

6. The compound of claim 1 wherein $R^1 = CH_2OH$;

R^3 is selected from the group consisting of CO_2R^{20} , $-CONR^7R^8$ and aryl; wherein the aryl substituent is optionally substituted with from 1 to 3 substituents independently selected

from the group consisting of halo, C₁₋₆ alkyl, CF₃, CN, OR²⁰, and CON(R²⁰)₂;

R⁷ is selected from the group consisting of hydrogen, C₁₋₁₀ alkyl and aryl, wherein the alkyl and aryl substituents are optionally substituted with from 1 to 2 substituents independently selected from the group consisting of halo, aryl, CF₃, CN, OR²⁰ and wherein each optional aryl substituent is optionally substituted with halo, alkyl, CF₃, CN, and OR²⁰;

R⁸ is selected from hydrogen and C₁₋₁₅ alkyl; and

R²⁰ is selected from hydrogen and C₁₋₄ alkyl.

7. The compound of claim 1 wherein R¹ = CH₂OH;

R³ is selected from the group consisting of CO₂R²⁰, -CONR⁷R⁸ and aryl wherein the aryl substituent is optionally substituted with from 1 to 2 substituents independently selected from the group consisting of halo, C₁₋₆ alkyl, CF₃ and OR²⁰;

R⁷ is selected from the group consisting of hydrogen, and C₁₋₈ alkyl, wherein the alkyl substituent is optionally substituted with one substituent selected from aryl, CF₃, CN, and OR²⁰ and wherein each optional aryl substituent is optionally substituted with halo, alkyl, CF₃, CN, or OR²⁰;

R⁸ is selected from hydrogen and C₁₋₈ alkyl; and

R²⁰ is selected from hydrogen and C₁₋₄ alkyl.

8. The compound of claim 1 wherein R¹ = CH₂OH;

R³ is selected from the group consisting of CO₂R²⁰, -CONR⁷R⁸, and aryl that is optionally substituted with from 1 to 2 substituents independently selected from the group of halo, C₁₋₃ alkyl, CF₃ and OR²⁰;

R⁷ is selected from the group consisting of hydrogen, and C₁₋₅ alkyl, wherein the alkyl substituent is optionally substituted with aryl, and wherein each optional aryl substituent is optionally substituted with halo, alkyl, CF₃;

R⁸ is selected from hydrogen and C₁₋₃ alkyl; and

R²⁰ is selected from hydrogen and C₁₋₄ alkyl.

9. The compound of claim 1 wherein R¹ = CH₂OH;

R³ is selected from the group consisting of CO₂R²⁰, -CONR⁷R⁸, and aryl that is optionally substituted with one substituent selected from the group of halo, C₁₋₃ alkyl, and OR²⁰;

R⁷ is selected from the group consisting of hydrogen, and C₁₋₅ alkyl, wherein the alkyl substituent is optionally substituted with aryl, and wherein each optional aryl substituent is optionally substituted with halo;

R⁸ is hydrogen; and

R^{20} is selected from hydrogen and C_{1-4} alkyl.

10. The compound of claim 1 wherein $R^1 = CH_2OH$;

R^3 is selected from the group consisting of CO_2R^{20} , $-CONR^7R^8$, and aryl that is optionally substituted with one substituent selected from halo, C_{1-3} alkyl and OR^{20} ;

5 R^7 is selected from the group consisting of hydrogen, and C_{1-5} alkyl, wherein the alkyl substituent is optionally substituted with aryl, and wherein each optional aryl substituent is optionally substituted with halo;

R^8 is hydrogen; and

R^{20} is selected from hydrogen and C_{1-4} alkyl.

10 11. The compound of claim 10 wherein R^7 is a methyl.

12. The compound of claim 10 wherein R_3 is $-CO_2Et$.

13. The compound of claim 1 wherein $R^1 = -CONHET$;

R^3 is selected from the group consisting of CO_2R^{20} , $-CONR^7R^8$, and aryl; that is optionally substituted with from 1 to 3 substituents independently selected from the group
15 consisting of halo, C_{1-6} alkyl, CF_3 , CN, OR^{20} , and $CON(R^{20})_2$;

R^7 is selected from the group consisting of hydrogen, C_{1-10} alkyl and aryl, wherein the alkyl and aryl substituents are optionally substituted with from 1 to 2 substituents independently selected from the group consisting of halo, aryl, CF_3 , CN, and OR^{20} and wherein each optional aryl substituent is optionally substituted with halo, alkyl, CF_3 , CN, and
20 OR^{20} ;

R^8 is selected from hydrogen, and C_{1-15} alkyl; and

R^{20} is selected from hydrogen, and C_{1-4} alkyl.

14. The compound of claim 1 wherein $R^1 = -CONHET$;

R^3 is selected from the group consisting of CO_2R^{20} , $-CONR^7R^8$, aryl that is optionally substituted with from 1 to 2 substituents independently selected from the group consisting of
25 halo, C_{1-6} alkyl, CF_3 and OR^{20} ;

R^7 is selected from the group consisting of hydrogen, C_{1-8} alkyl, and aryl, wherein the alkyl and aryl substituents are optionally substituted with one substituent selected from the group consisting of halo, aryl, CF_3 , CN, OR^{20} and each optional aryl substituent is optionally
30 substituted with halo, alkyl, CF_3 , CN, and OR^{20} ;

R^8 is selected from hydrogen, and C_{1-8} alkyl; and

R^{20} is selected from hydrogen, and C_{1-4} alkyl.

15. The compound of claim 1 wherein $R^1 = -CONHET$;

R^3 is selected from the group consisting of CO_2R^{20} , $-CONR^7R^8$, and aryl that is

optionally substituted with from 1 to 2 substituents independently selected from the group consisting of halo, C₁₋₃ alkyl, CF₃ and OR²⁰;

R⁷ is selected from the group consisting of hydrogen, and C₁₋₅ alkyl, wherein the alkyl substituent is optionally substituted with aryl, and wherein each optional aryl substituent is optionally substituted with halo, alkyl, CF₃;

R⁸ is selected from hydrogen, and C₁₋₃ alkyl; and

R²⁰ is selected from hydrogen, and C₁₋₄ alkyl.

16. The compound of claim 1 wherein R¹ = -CONHEt;

R³ is selected from the group consisting of CO₂R²⁰, -CONR⁷R⁸, and aryl that is optionally substituted with one substituent selected from halo, C₁₋₃ alkyl and OR²⁰;

R⁷ is selected from the group consisting of hydrogen, and C₁₋₅ alkyl, wherein the alkyl substituent is optionally substituted with aryl, and wherein each optional aryl substituent is optionally substituted with halo;

R⁸ is hydrogen; and

R²⁰ is selected from hydrogen, and C₁₋₄ alkyl.

17. The compound of claim 1 wherein R¹ = -CONHEt;

R³ is selected from the group consisting of CO₂R²⁰, -CONR⁷R⁸, and aryl that is optionally substituted with one substituent selected from halo, C₁₋₃ alkyl and OR²⁰;

R⁷ is selected from hydrogen, and C₁₋₃ alkyl;

R⁸ is hydrogen; and

R²⁰ is selected from hydrogen, and C₁₋₄ alkyl.

18. The compound of claim 10 where R¹ is -CONHEt.

19. A compound matter of claim 1 wherein the compound is selected from ethyl-
{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-
yl}pyrazole-4-carboxylate, (4S,2R,3R,5R)-2-{6-amino-2-[4-(4-chlorophenyl)-
pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3,4-diol, (4S,2R,3R,5R)-2-{6-amino-2-[4-
(4-methoxyphenyl)pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3,4-diol, (4S,2R,3R,5R)-
2-{6-amino-2-[4-(4-methylphenyl)pyrazolyl]purin-9-yl}-5-(hydroxymethyl)-oxolane-3,4-diol,
(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-
yl}pyrazol-4-yl)-N-methylcarboxamide, 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-
(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylic acid, (1-{9-
[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-
yl)-N,N-dimethylcarboxamide, (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-
(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-ethylcarboxamide, 1-{9-

[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxamide, 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-(cyclopentylmethyl)carboxamide, (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-[(4-chlorophenyl)methyl]carboxamide, Ethyl 2-[(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)carbonylamino]acetate, and mixtures thereof.

20. A method for stimulating coronary vasodilatation in a mammal by administering to the mammal a therapeutically effective amount of a compound of claim 1 that is sufficient to stress the heart and induce a coronary steal situation for the purposes of imaging the heart.

21. The method of claim 20 wherein the therapeutically effective amount ranges from about 0.01 to about 100 mg/kg weight of the mammal.

22. The method of claim 20 wherein the mammal is a human.

23. A pharmaceutical composition comprising the compound of claim 1 and one or more pharmaceutical excipients;

24. The pharmaceutical composition of claim 23 wherein the pharmaceutical composition is in the form of a solution.

25. The pharmaceutical composition of claim 23 wherein the composition is useful as an anti-inflammatory, in adjunctive therapy with angioplasty, as a platelet aggregation inhibitor, and as an inhibitor of platelet and neutrophil activation.

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(54) Title: N-PYRAZOLE A₂A RECEPTOR AGONISTS

(57) Abstract: 2-Adenosine N-pyrazole compounds having formula (1) and methods for using compounds as A₂A receptor agonists to stimulate mammalian coronary vasodilatation for therapeutic purposes and for purposes of imaging the heart.

WO 00/78779 A3

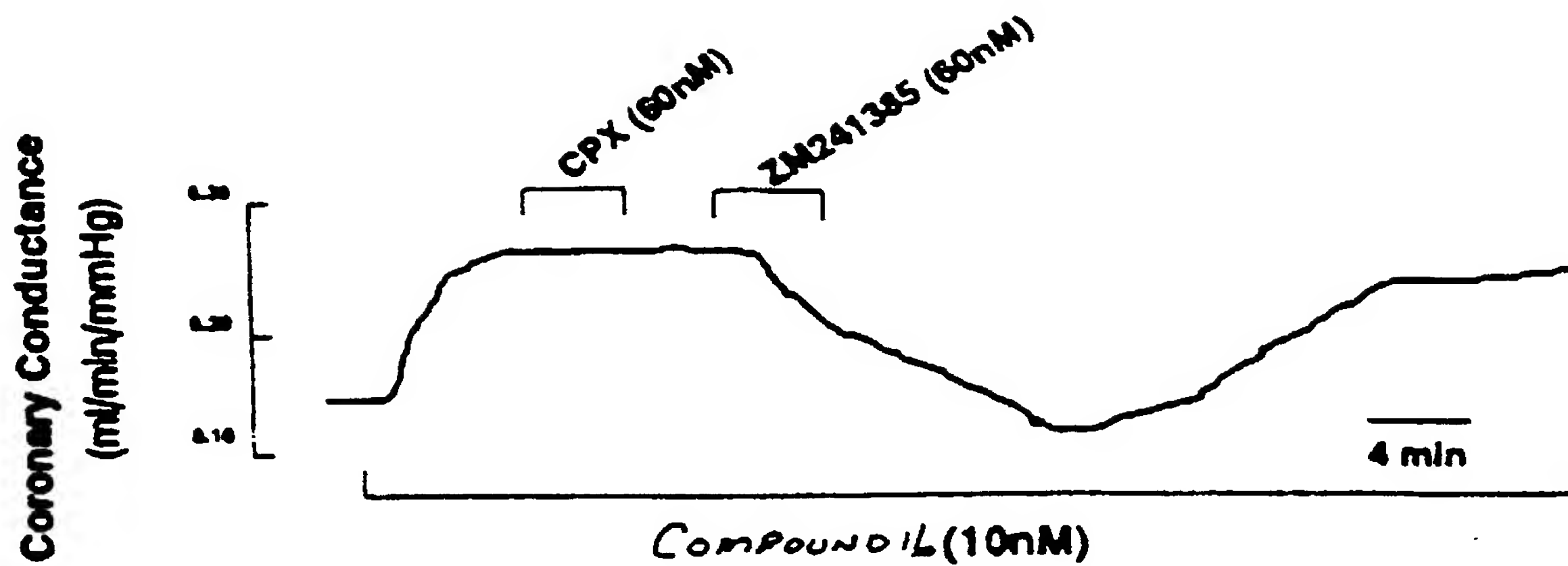


Figure 1A

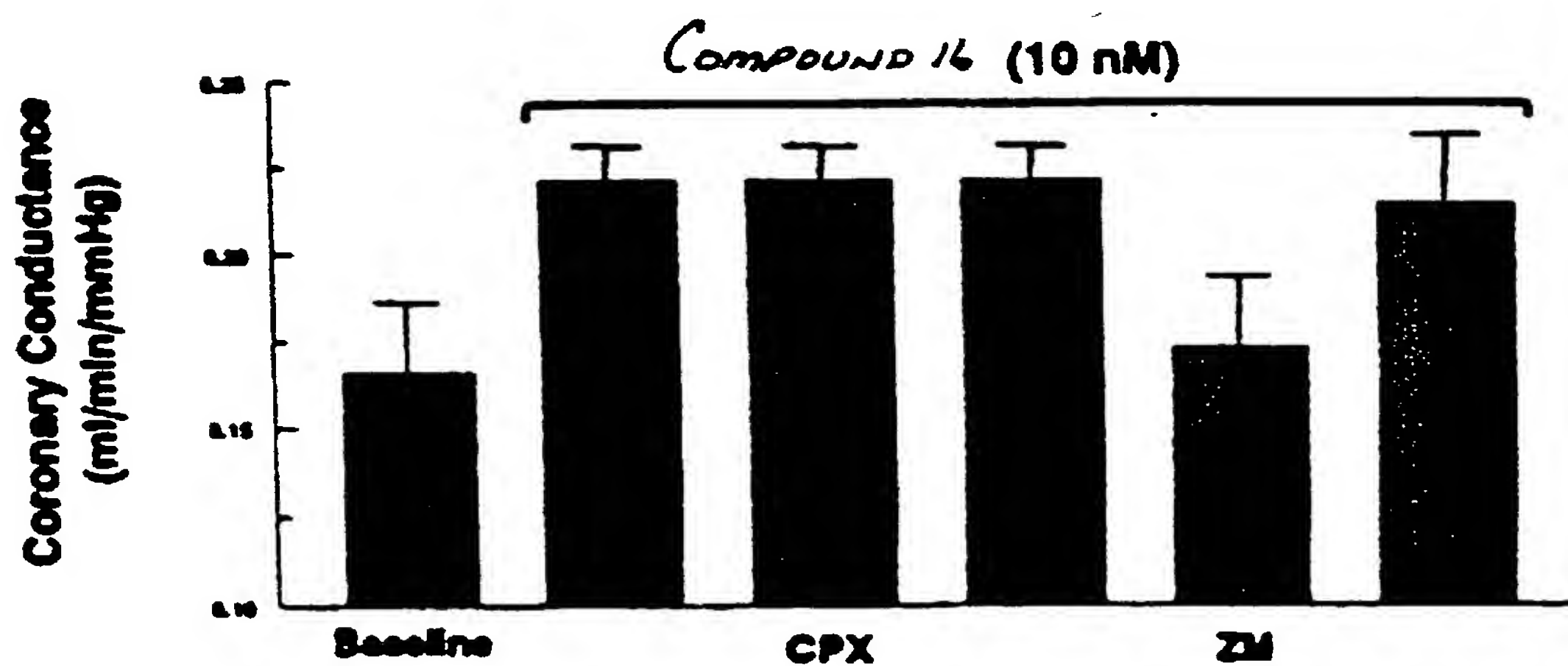


Figure 1B

10/018446

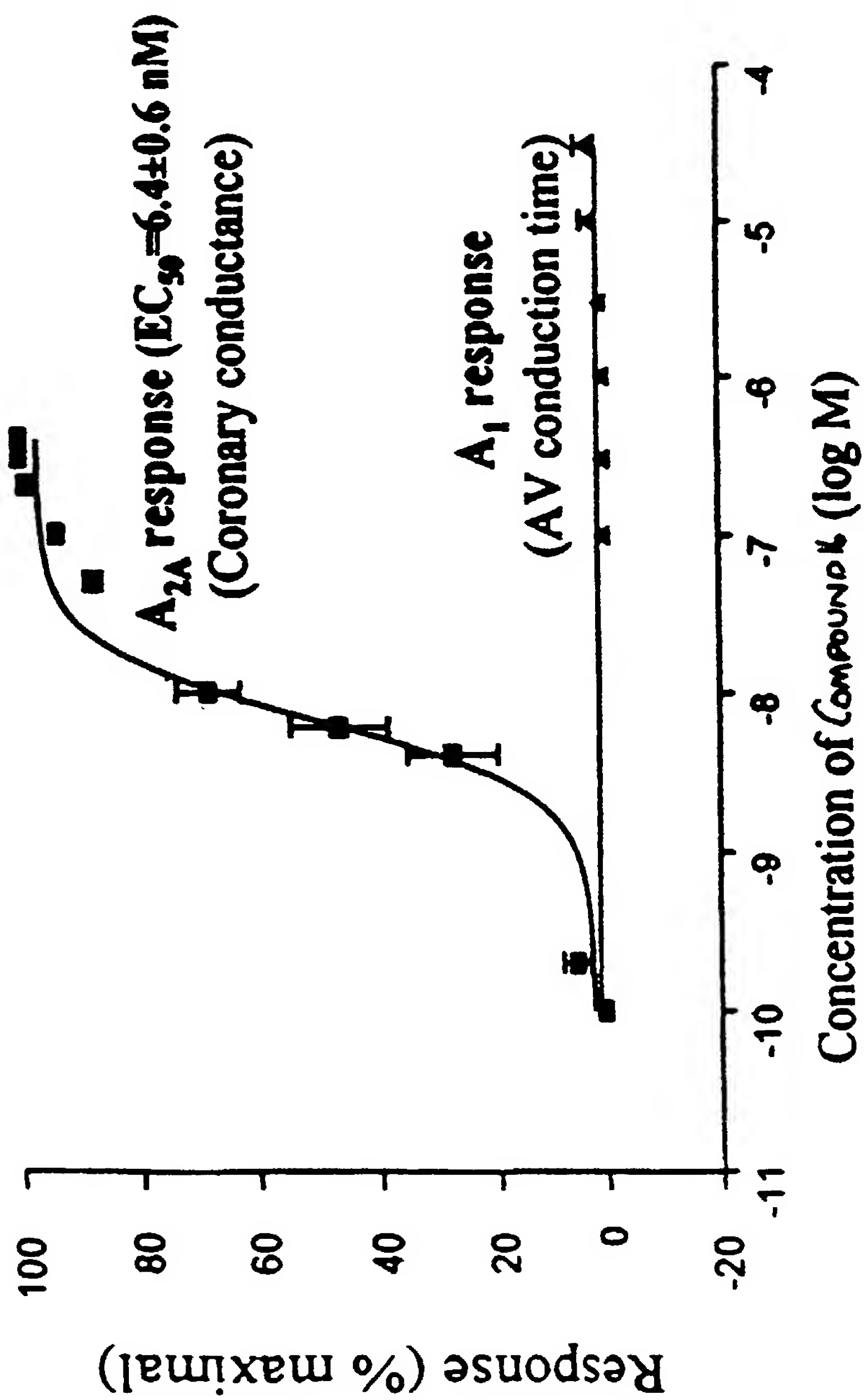


Figure 2

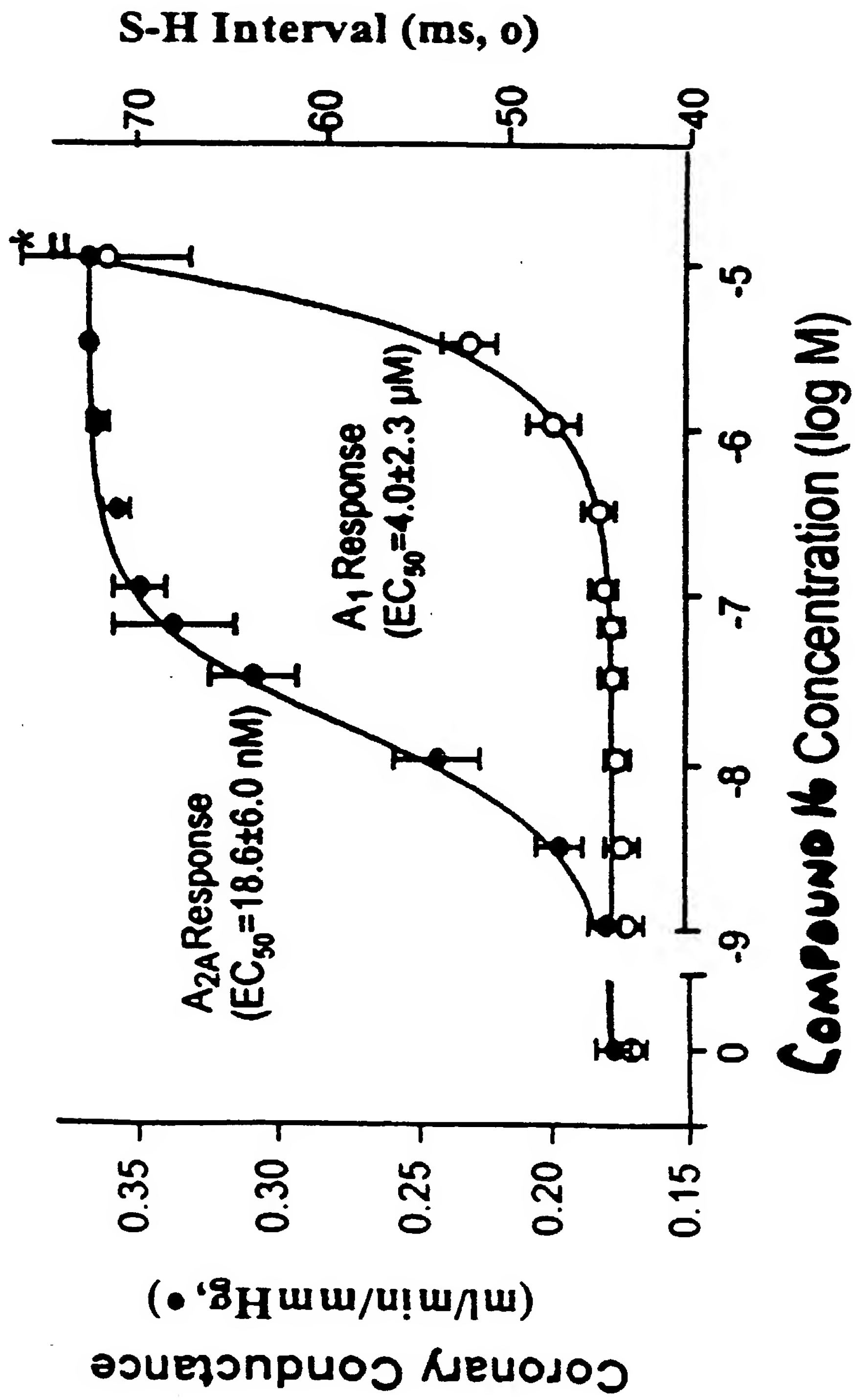


Figure 3

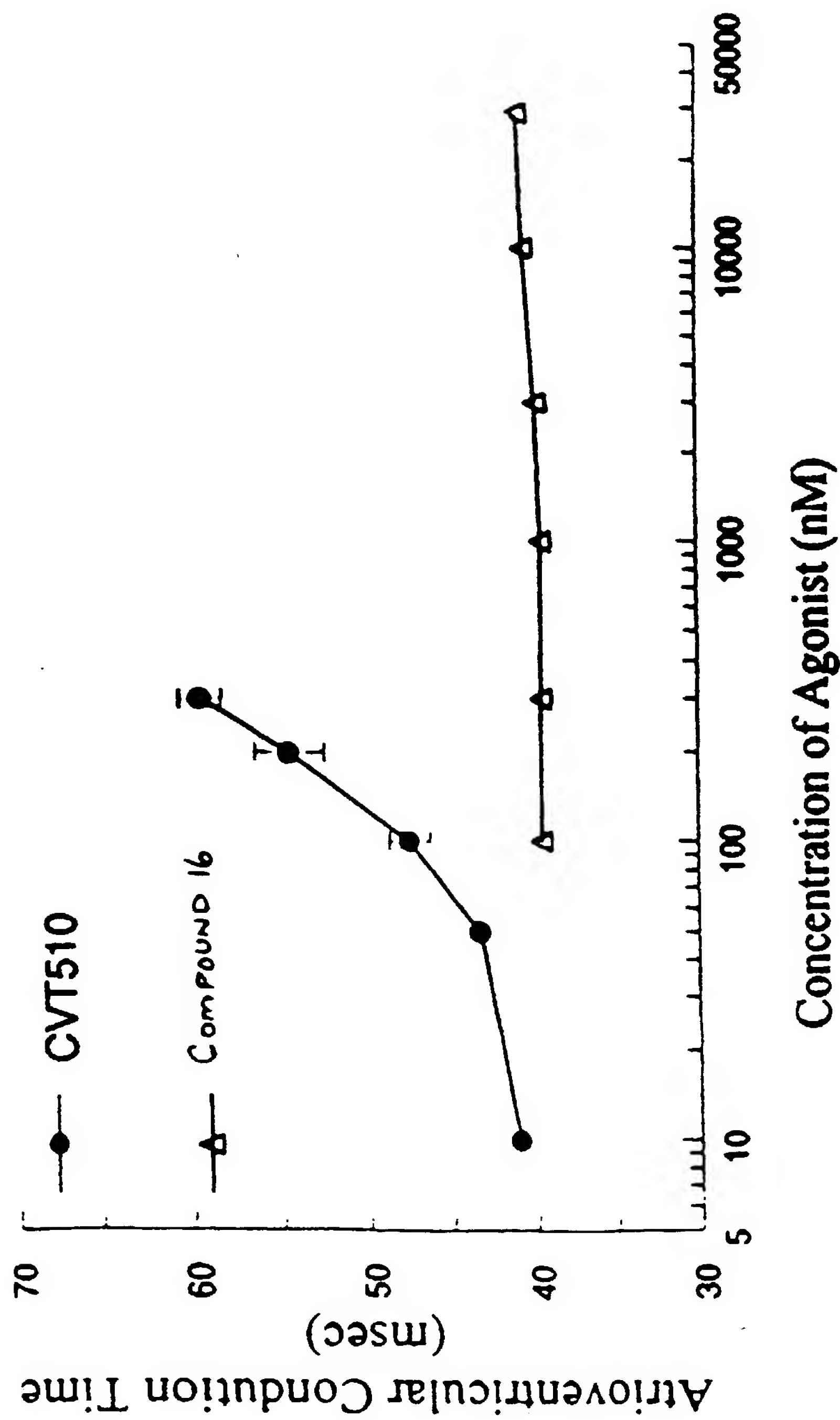


Figure 4

Case No.: 99,423-S

**DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

N-Pyrazole A2A Receptor Agonists

the specification of which is attached hereto unless the following space is checked:

☐ was filed on _____ as United States Application Serial Number _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s):

	<u>Number</u>	<u>Country</u>	<u>Day/Month/Year Filed</u>
1.	PCT/US00/40281	PCT	21 June 2000
2.			

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

	<u>Application Number</u>	<u>Filing Date</u>	<u>Status: patented, pending, abandoned</u>
1.	09/388,185	22 June 1999	Pending
2.			

I hereby appoint the practitioners associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and I direct that all correspondence be addressed to that Customer Number.

Customer Number: **020306**

Principal attorney or agent: A. Blair Hughes

Telephone number: 312-913-0001

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that

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